Review: IgA anaphylactic transfusion reactions. Part I. Laboratory diagnosis, incidence, and supply of IgA-deficient products

R.R. VASSALLO

Despite yielding a definitive diagnosis in fewer than 20 percent of anaphylactic transfusion reactions, investigation for IgA deficiency and the presence of presumably pathogenic IgG anti-IgA is useful in patient management. Individuals with demonstrated anti-IgA are thereafter committed to receiving IgA-depleted cellular products or IgA-deficient plasma and derivatives to prevent recurrent severe reactions. Unfortunately, in populations of IgA-deficient individuals screened for anti-IgA, the predictive value of the test in the absence of a prior reaction is quite low. Anti-IgA testing is complex and limited to a few reference laboratories, many of which still employ a labor-intensive hemagglutination assay developed in the late 1960s. Timely decisions regarding further transfusion management of patients experiencing anaphylaxis often rely upon more rapidly obtained assays of the IgA concentration as an indicator of the likelihood of subsequent demonstration of anti-IgA. The scarcity of IgA-deficient banked plasma products and dedicated plateletpheresis donors has led to the development of American Rare Donor Program policies designed to appropriately allocate these precious resources. The test methods used to establish the diagnosis of IgA deficiency and identify the approximately one-third of these individuals with anti-IgA are discussed, along with the incidence of abnormal tests in various populations. Also presented are testing recommendations for the identification of an IgA-mediated mechanism for transfusion-associated anaphylaxis and qualification of patients to receive rare IgA-deficient plasma-containing products. *Immunohematology* 2005;21:226–233.

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The diagnosis of an IgA-related anaphylactic reaction requires a clinical diagnosis that is confirmed by specialized assays for IgA concentration and anti-IgA. This review will address these laboratory assays, their application to defining the incidence of IgA-related transfusion reactions, and the provision of IgA-deficient blood products. In the accompanying review, Sandler and Zantek address the clinical diagnosis of IgA anaphylactic reactions and bedside management.

**The Role of IgA in Major Transfusion Reactions**

Numerous well-documented mechanisms exist by which blood products can trigger major allergic reactions (i.e., anaphylaxis or severe anaphylactoid reactions). Besides unrecognized coincidental events unrelated to the transfusion, these mechanisms include: administration of blood containing antigens to which the recipient is presensitized (e.g., medications, foods, or chemicals like latex or plasticizers), infusion of biological mediators produced during product storage, transfer of donor IgE antibodies against substances in the recipient's blood, transfusion of presensitized individuals with polymorphic forms of common serum proteins (e.g., haptoglobin [especially in Japanese populations], transferrin, α1-antitrypsin, albumin, etc.), and, central to this review, exposure of recipients with preformed antibodies to transfused IgA. These preformed antibodies may be class-specific (anti-IgA), subclass-specific (anti-IgA1 or -IgA2), or allotype-specific (anti-IgA2m[1] or -IgA2m[2]). Subclass- and allotype-specific antibodies are frequently referred to as being of “limited specificity.” The most severe reported reactions tend to result from class-specific antibodies in severely IgA-deficient patients. Limited-specificity antibodies have been reported to be associated with less severe reactions,
often in patients with low or even normal concentrations of IgA.

Sandler et al.° reported that only 18.1 percent of 359 sera from patients with anaphylactic or anaphylactoid transfusion reactions contained anti-IgA of any specificity by passive hemagglutination assay. Clearly, analogous to the old medical dictum, “not all that wheezes is asthma,” not all that anaphylaxes during transfusion is IgA-related. A robust risk estimate of the incidence of transfusion-associated major allergic reactions comes from the Québec Hemovigilance system.° For RBC transfusions, the reported risk was 4.3 per 100,000 units, and for platelet transfusions, 62.6 per 100,000 platelet pools. Domen and Hoeltge found 10.4 severe reactions per 100,000 platelet, 3.5 per 100,000 plasma, and 1.7 per 100,000 RBC transfusions in a retrospective review at the Cleveland Clinic.° The reported rate of IgA-related major allergic reactions to RBCs from single institutions is 1.7 per 100,000° and 2.1 per 100,000.° These rates are not inconsistent with Sandler et al.’s finding, as one of the four cases used to generate the higher of the two institutional rates occurred in the setting of an urticarial reaction (estimated frequency 400 per 100,000 RBC transfusions),° which no doubt was reported because of a coincidental myocardial infarction. Furthermore, and more importantly, the true risk estimates from Québec (and Ohio) are likely somewhat higher due to underrecognition and underreporting of transfusion reactions.

The exact cause for most major allergic reactions often goes undetermined.° Nevertheless, the proven ability of washed cellular or IgA-deficient cellular or plasma products to prevent recurrent reactions in recipients with previous reactions mandates a prompt, carefully planned investigation for the presence of pathogenic antibodies. Many advocate the screening of patients at high risk for development of anti-IgA before blood products or derivatives are administered, to prevent anaphylaxis.° This latter approach would obviously be important when, not uncommonly, one considers prophylactic use of IgA-containing IVIG for frequent infections or therapeutic use in coexisting autoimmune diseases. Others note that detection of anti-IgA by passive hemagglutination does not necessarily predict risk for anaphylactic reactions.° This screening approach may then unneccessarily commit patients with positive tests to a lifelong policy of IgA-deficient plasma transfusion and could result in harmful delays. The development of anti-IgA assays that more reliably distinguish individuals at risk for major allergic reactions is therefore of paramount importance.

**Epidemiology of IgA Deficiency**

Selective IgA deficiency (i.e., IgA only) is the most common primary immunodeficiency in the Western World.° In up to 20 percent of individuals, IgA deficiency may be accompanied by an IgG subclass deficiency, most commonly affecting IgG2 ± IgG4,° or may be part of a broader condition, common variable immunoglobulin deficiency, characterized by total absence of IgG, IgA, and, sometimes, IgM. The clinical definition of selective IgA deficiency has been recently revised to a somewhat higher serum IgA concentration (< 7 mg/dL in individuals > 4 years old)° than that previously advocated by the World Health Organization (< 5 mg/dL). Up to 80 percent of these individuals are at risk of developing sinopulmonary infections or allergies of variable clinical significance, as well as autoimmune disorders and malabsorption syndromes, within 20 years of diagnosis.° “Severe IgA deficiency,” however, has been variously defined as < 0.5 mg/dL,°< 0.16 mg/dL,° or < 0.05 mg/dL.° The American rare donor program uses the < 0.05 mg/dL level to classify its donors as IgA-deficient (A. Church, personal communication). This assures that products provided to patients sensitized to IgA contain far lower concentrations of IgA than those implicated in the development of major allergic reactions.° Class-specific anti-IgA develop in individuals with the very lowest concentrations of IgA. As stated above, individuals who have relatively low or even normal concentrations but who lack an IgA subclass or allotype may develop limited-specificity antibodies, reported to be capable of inducing allergic reactions upon IgA challenge, almost all of which are minor reactions. The incidence of IgA deficiency reported in the literature varies not only with the population studied, but also with the detection limits of the screening test. In the United States, 1 in 328 blood donors had an IgA concentration below 5 mg/dL by immunodiffusion assay.° International estimates using similarly sensitive assays for IgA concentration include 1 in 168 unselected Spanish children,° 1 in 406 Czech blood donors,° and 1 in 442 Australian blood donors,° but only 1 in 4100 Chinese° and 1 in 18,500 Japanese blood donors.° Identification of individuals whose plasma contains such low concentrations of IgA (< 0.05 mg/dL) that they may donate products for
sensitized patients requires assays not routinely available outside of specialized laboratories. Using more sensitive screening assays, incidence estimates in the same or similar populations reveal that roughly half of those found to be IgA deficient have plasma concentrations low enough to qualify them as IgA-deficient donors. Among blood donors, 1 in 507 Finns, 1 in 875 English, and 1 in 886 Americans qualify, but as only 1 in 93,000 Japanese manifested concentrations this low, establishment of an IgA-deficient donor registry in that country has been quite challenging.

Principles of Testing for IgA-Mediated Transfusion Reactions

The definitive diagnosis of an IgA-mediated allergic reaction requires the demonstration of anti-IgA. However, because the most severe allergic reactions usually occur in the setting of class-specific antibodies in severely IgA-deficient individuals, and quantitative IgA assays are faster and more widely available than anti-IgA assays, many algorithms for the evaluation of anaphylaxis begin with a determination of pretransfusion serum IgA concentrations. (A pretransfusion specimen is necessary because transfused plasma would be expected to contain normal concentrations of IgA, artificially elevating posttransfusion measurements). The identification of severe IgA deficiency in a patient who suffered a major allergic reaction vastly increases the likelihood of a role for anti-IgA in the reaction. Sandler et al. found that among 359 individuals experiencing a major allergic transfusion reaction, 76.3 percent with serum IgA < 0.05 mg/dL had class-specific IgA antibodies. A low or normal IgA concentration cannot entirely rule out a role for anti-IgA in an allergic reaction, since antibody assays may demonstrate a limited-specificity antibody in up to 0.7 percent of patients. These antibodies are of uncertain clinical significance, since the majority of reported reactions with limited-specificity antibodies are urticarial, not anaphylactic. Table 1 lists some of the available methods to determine serum IgA concentrations, their sensitivity, and the length of time required to perform them.

Quantitation of IgA

Three basic principles underlie assays of plasma IgA concentrations. The first involves quantitation through analysis of precipitates or light-scattering characteristics of IgA:anti-IgA immune complexes. The second utilizes hemagglutination as an end point of the antigen:antibody reaction, the third uses specifically labeled antibodies to measure IgA concentrations. In the immune complex-based test category, immuno-diffusion, immunoelectrophoretic, and nephelometric/turbidometric assays are available. Ouchterlony double diffusion employs an agar plate whose central well is filled with anti-human-IgA. Serum or plasma samples are placed in surrounding wells and absence of a precipitin arc signifies sample concentrations below the cutoff established by the antibody and sample dilutions used. This test and immunoelectrophoresis are the least sensitive quantitation techniques, best suited to screening individuals for further testing. (Immunoelectrophoresis is usually used in searching for high concentrations of paraproteins, but it can identify levels in the lower ranges.) Low-level radial immunodiffusion kits are available commercially; they can also identify patients requiring further testing to distinguish those with the very lowest concentrations of IgA who may have formed class-specific IgA antibodies. All three technologies require extended plate incubation before test results are available. Nephelometers and turbidometers provide faster results but are not uniformly available in the hospital setting. Both project light through a sample cuvette in which IgA standards or patient test samples are reacted with a known concentration of anti-human-IgA. Nephelometers measure the light scattered through the sample at an angle from the incident beam, while less sensitive turbidometers measure direct light attenuation as antigen-antibody complexes form in solution. Both compare these to the light-extinguishing dilutions of IgA standards to derive a measured concentration. Rate nephelometers improve upon the sensitivity of the technique by determining the rate of
change of light scattering rather than static values. All of these techniques, if rapidly available at the point of patient care, may increase the probability of detecting an anti-IgA mechanism for major allergic transfusion reactions by defining a population of individuals more likely to have extremely low concentrations of IgA and, thus, class-specific anti-IgA.

In an effort to devise a rapid, more sensitive screening technique for IgA deficiency, Schulenburg et al. 27 developed a solid phase RBC adherence assay comparable in sensitivity to but faster than radial immunodiffusion and more widely available than rate nephelometry. Others have found the assay technically difficult to establish, 15 and the test is not commercially available.

The use of highly sensitive immunoassays to quantitate plasma IgA is limited to specialized reference laboratories that frequently cannot provide rapid results when immediate postanaphylaxis transfusion decisions must be made. They are able to distinguish individuals with plasma IgA concentrations below 0.05 mg/dL (i.e., severely IgA-deficient individuals). Highly sensitive quantitation of IgA in patients experiencing anaphylactic transfusion reactions is most useful if anti-IgA assays would introduce intolerable delays in patient care and faster, less sensitive screening studies are unavailable. In nonemergent situations, however, use of rare IgA-deficient products is unwarranted without first demonstrating circulating anti-IgA. High sensitivity studies are also an absolute requirement to establish a panel of IgA-deficient blood donors. One of the first assays developed for high-sensitivity quantitation of IgA was the passive hemagglutination inhibition assay. 28 Still in use today, this method is adaptable to automated high volume blood donor screening on Olympus PK or Groupomatic platforms. 29,30 In this assay, group O negative RBCs are coated with purified IgA using tannic acid or chromic chloride. Test sera are incubated with a diluted anti-human-IgA and then added to a suspension of RBCs in microplate wells. After centrifugation, wells with visible agglutination of the test cells indicate sera with very low concentrations of IgA insufficient to neutralize the anti-human-IgA. Because the assay uses RBC agglutination as an end point, anti-RBC antibodies in test sera invalidate the assay. More and more, highly sensitive enzyme-linked immunoassays are supplanting this technically demanding test. The most sensitive technique, radioimmunoassay, is costly, involves use of radionuclides, and has been largely replaced by ELISA. Several ELISA techniques have been described, but as yet no FDA-approved commercial kits are available; and these tests must be locally developed and validated. 31,32

Anti-IgA and Its Demonstration

Reported rates of what percentage of at-risk IgA-deficient individuals actually develop IgG or IgM anti-IgA depend upon the primary immunodeficiency state studied and, in some cases, concurrent disease states. For otherwise healthy, selective-IgA-deficient individuals, antibodies have been identified in 36.8 percent of those with IgA concentrations < 5 mg/dL, 33 and 28 percent, 14 28.8 percent, 3 and 29 percent 34 of those with concentrations < 0.05 mg/dL. Interestingly, in individuals with combined IgA and IgG2 deficiency, 60 percent of tested subjects had antibody, 34 as did 50 percent of selective-IgA-deficient patients whose clinical course was complicated by rheumatoid arthritis (RA), 77 percent of those with juvenile RA, and 100 percent of those with systemic lupus erythematosus. 35 Seventy-three percent of IgA-deficient individuals with ataxia-telangiectasia were demonstrated to have anti-IgA. 35 These data collectively indicate that a clinically recognizable state of immune dysregulation superimposed upon selective IgA deficiency is associated with the formation of anti-IgA and that the application of anti-IgA assays to individuals with progressively higher concentrations of IgA probably picks up additional, limited-specificity antibodies in those with IgA subclass, or allotype deficiencies, or IgA autoanti-bodies. A frequently stated estimate of the detection of anti-IgA in approximately one-third of IgA-deficient individuals is dependent upon both the composition of the IgA-deficient population as well as the sensitivity of the various methods used to demonstrate antibodies. 36

The commonly misquoted rate of 1 in 20,000 to 1 in 47,000 for IgA-related major transfusion reactions comes from the limited data reported by Bjerrum and Jersild (3 reactions, only 1 of which was actually anti-IgA-related in ~60,000 transfusion events) 4 and Pineda and Taswell (4 anti-IgA-related cases in ~188,000 transfusions). 7 The true rates are therefore 1 in 47,000 to 1 in 60,000, or a combined rate of ~1 in 50,000. All five patients reacted to whole blood or packed cells and, while this is not explicitly stated, are presumably compared to a RBC transfusion denominator. Therefore, while 1 in 1200 Americans has IgA deficiency with co-existing anti-IgA, the rate of anti-IgA-mediated
reactions to RBC transfusions is almost 42 times less frequent, i.e., a major allergic reaction may occur in only 2.4 percent of those with identified antibodies! Clearly, we are currently unable to identify what feature of these antibodies truly defines their pathogenicity. The importance of the isotype of anti-IgA also remains unresolved. A 1986 Duke University study detected IgE anti-IgA by ELISA in 9 of 18 patients with allergic transfusion reactions, two of whom had suffered unmistakable anaphylactic events. However, Mayo Clinic researchers using an immunoradiometric assay (IRMA) were unable to detect IgE anti-IgA by IRMA and passive hemagglutination. IgE antibodies were similarly unreported in a series of patients with anaphylactic transfusion reactions in the setting of selective IgA deficiency. Methods to reliably distinguish clinically relevant anti-IgA have yet to be identified. As a result, recommendations cannot be made regarding which individuals with anti-IgA are able to safely receive IgA-containing plasma products or how often patients with previously identified severe IgA deficiency should be screened for antibody formation.

Despite the shortcomings of anti-IgA studies in predicting who is likely to experience a major allergic reaction, a positive result in any of the several available assays is a requirement before elective release of rare IgA-deficient plasma. Available testing methods are listed in Table 2. The first test developed was the passive hemagglutination assay (PHA). It remains a sensitive and reliable, but time-consuming and technically demanding, method available in a small number of reference labs. The assay involves coating group O, D– RBCs with purified myeloma proteins using tannic acid or chromic chloride. Tannic acid-treated RBCs are stable for longer periods (14 days versus 2 for chromic chloride–treated cells) but may manifest nonspecific serum reactivity to “tanned cells.” Separate cells are coated with IgA1, IgA2m(1), and IgA2m(2), and two different sources of each protein are included in the panel. Diluted test sera are added to V-bottom microplates and observed for hemagglutination following centrifugation. Class-specific antibody is present when all six examples of IgA-coated RBCs agglutinate. Antibody subclass specificity to IgA1 or IgA2 can be determined by reaction with only IgA1- or IgA2-coated cells, while allotype-specific antibodies react only with the two examples of IgA2m(1) or IgA2m(2). Specificity is confirmed by neutralization with purified total IgA or IgA of the appropriate subclass or allotype. Antibody reactivity may also be titrated using serial sample dilutions. Major allergic reactions are often associated with class-specific antibodies of high titer (> 1:1,000); these antibody levels have been reported to remain quite stable over a median follow-up period of 19 years. Sandler et al. reported that the correlation of clinical severity of reactions and antibody titers was not good, possibly because in the wake of an acute reaction, some of the antibody may be removed in complexes with infused IgA. The assay detects hemagglutinating antibodies (IgM and IgG), but cannot identify IgE antibodies. Invalid runs may also occur when reactivity is seen without clear class, subclass, or allotype distinction, due either to nonspecific antibody reactions with storage-induced IgA neo-epitopes or to variable specific reactivity to different IgA myeloma proteins of the same subclass or allotype. The test cannot be interpreted in the presence of RBC antibodies, recognized by their reactivity with uncoated control RBCs. Selection of antigen-negative RBCs for IgA coating can prevent this false reactivity.

A flow cytometry–based microbead immunoassay has been described, using IgA-coated polystyrene beads which are incubated with serum samples. A FITC-conjugated anti-human IgG causes fluorescence of beads incubated with test sera containing anti-IgA. Fluorescence is detected and anti-IgA is quantitated using a standard flow cytometer. The requirement for a dedicated flow cytometry facility has limited the applicability of this assay.

Solid phase immunoradiometric assays utilizing IgA coupled to microcrystalline cellulose are available. Test sera are incubated overnight with the cellulose-IgA reagent and probed for anti-IgA binding with radiolabeled anti-human IgG using a gamma counter. A number of reference laboratories offer this test, which reliably detects class-specific and, somewhat less reliably so, limited-specificity antibodies. Enzyme immunoassays, which incubate test samples in

Table 2. Available anti-IgA assays

| Passive hemagglutination assay (PHA) |
| Immunoradiometric assay (IRMA) |
| Flow cytometry microbead immunoassay (MIA) |
| Enzyme-linked immunosorbent assay (ELISA) |
| Particle gel immunoassay (PaGIA) |

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IgA-coated microplates, are available. Anti-IgA is detected using enzyme-conjugated anti-human IgG and binding specificity is confirmed by neutralization with the IgA preparation used to coat the plates. The ease of this method over the other previously described methods offers advantages in speed and efficiency in reference labs offering anti-IgA studies. ELISA techniques may not be as sensitive in detecting limited-specificity antibodies as a passive hemagglutination assay (PHA), possibly due to the lower concentrations of IgA2 in polyclonal IgA preparations (personal observation).

A new detection technology based on a standard particle-gel immunoassay technique (PaGIA) is under development. Diluted test samples are incubated with red polystyrene beads coated with IgA in the reaction chamber on an ID-MicroTyping System card just above an anti-human IgG gel-containing microwell. Sera containing anti-IgA result in agglutination of the beads, which become trapped in or above the gel during centrifugation. Unagglutinated beads (i.e., those in wells containing test sera without anti-IgA) travel through the gel particles and form a visible pellet at the bottom of the microtube. Results are read macroscopically. Very little is available in the literature about this technology, which, if found to be sufficiently sensitive, is rapid and uses equipment available in many blood banks. One study found the technique sensitive to high-titer (> 1:1,000) anti-IgA demonstrated by passive hemagglutination techniques, but less so for lower titer antibodies. No false positives were seen in either study.

Testing Algorithms and Qualification for Rare Donor Plasma Products

Because of the known risks of re-exposing individuals who have experienced IgA-related anaphylactic transfusion reactions to IgA-containing blood products, cellular blood products are washed to remove plasma. Plasma products, however, must be provided from IgA-deficient donors. The American Rare Donor Program (ARDP), described in this issue of Immunohematology, will identify locations of IgA-deficient frozen plasma and request shipment to authorized requesting U.S. blood centers and, rarely, those in other countries. All donors in their database are tested twice, using high-sensitivity IgA assays to ensure severe deficiency (i.e., < 0.05 mg/dL). Because of the limited supply of plasma, ARDP technical and medical staff carefully assess each request.

For elective and most nonemergent requests, ARDP policies generally require the demonstration of class-specific or limited-specificity anti-IgA in a patient’s serum, regardless of whether the patient has previously experienced an allergic transfusion reaction. Since the likelihood of non-IgA-related causes of anaphylactic transfusion reactions far outweighs the likelihood of relation to anti-IgA (only 18% in Sandler et al’s study), prior to the release of IgA-deficient plasma, a serum IgA concentration must be determined by the most expedient method. In an emergency, when IgA-antibody testing has not been completed, individuals without detectable IgA by either high-sensitivity or more rapidly available low-sensitivity testing (see test sensitivities in Table 1) will be provided with IgA-deficient plasma until antibody testing is found to be negative. Patients with low but detectable concentrations of IgA rarely have limited-specificity antibodies identified upon appropriate testing. In addition, since most, if not all, of the reactions associated with limited-specificity antibodies are minor ones, IgA-deficient plasma cannot be provided until antibody studies demonstrate subclass- or allotype-specific antibodies. In unusual circumstances, short-term distribution of IgA-deficient plasma may be required after direct communication between the patient’s physician and ARDP medical staff.

Since platelets are suspended in large volumes of plasma and platelet washing is technically demanding, the ARDP also maintains a list of blood centers that have IgA-deficient donors in their apheresis programs. ARDP staff can facilitate the recruitment of these donors when platelet washing is locally unavailable or patients continue to experience reactions with washed platelet units. Generally, IgA-deficient cryoprecipitate is not available through the ARDP.

Summary

The laboratory investigation of an anaphylactic transfusion reaction includes testing for anti-IgA. Washed or frozen, deglycerolized RBC transfusions need not be delayed during a laboratory investigation to determine the need for special IgA-deficient blood products. Due to the involved nature of antibody testing, surrogate assays for the absence of IgA may be completed within a time frame commensurate with making important clinical decisions regarding plasma or platelet transfusion. The testing requirements of the American Rare Donor Program, a valuable resource for rare plasma units, have been discussed.
Prophylactic testing for anti-IgA in patients with known IgA-deficiency who are anticipated to require blood transfusion or IVIG treatment is reasonable, but identifies many individuals who are not at risk for major allergic reactions, due to the poor predictive value of the assay. In addition, how often one should test individuals who have not formed antibody is unknown, though one study found that 11.8 percent of individuals with IgA concentrations < 0.05 mg/dL developed antibodies over a follow-up period of 16 to 21 years. The need for ongoing investigation to determine a better marker for allergic risk is clear and urgent.

References


Ralph R. Vassallo, Jr, MD, Senior Medical Director, American Red Cross Blood Services—Penn-Jersey Region, 700 Spring Garden Street, Philadelphia, PA