The investigation of the significance of a positive direct antiglobulin test in blood donors

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Sixty-two samples from 62 donors were investigated to determine the significance of warm IgG autoantibodies that were detected using a gel system during compatibility testing. The presence of autoantibodies on the red cells was confirmed by elution studies. Twelve of 23 strongly positive samples, 7 of 19 moderately positive samples, and 6 of 11 weakly positive samples were studied. The remaining nine samples were found positive during crossmatching, then negative when it was repeated. These nine samples were not included in this study. With a tube test, most of the antibodies had titers from 4 to 8. IgG subclass studies showed that 14 of 25 samples with reactive eluates contained IgG1, one contained IgG1+IgG2, one contained IgG1+IgG4, and two contained IgG1+IgG3 weak. The frequency of donors with a positive direct antiglobulin test (DAT) was ~ 1 in 3000 and males were twice as likely to be DAT positive (8 females vs. 17 males in this study). None of the donors had hemolysis. Two donors showed low-titer anticardiolipin antibodies. We conclude that DAT-positive donors can be a problem during compatibility testing when sensitive methods are used. ***Immunohematology* 2002;18:78–81.**

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A positive direct antiglobulin test (DAT) occurs occasionally in normal blood donors, and often is discovered when the donor's red cells are incompatible in a compatibility test. The frequency of donors with a positive DAT was estimated to be 1 in 9000 to 1 in 14,000.[1] In the majority of cases, long-term follow-up revealed no clinical condition that would account for the positive IgG DAT. IgG autoantibodies that are responsible for immune-mediated destruction of red blood cells (RBCs) in patients with warm-antibody autoimmune hemolytic anemia (AIHA) are predominantly IgG, subclass 1.[1,2] IgG3 autoantibodies, however, are most effective in producing RBC destruction. The presence of IgG3 on the RBCs of a patient without hemolytic anemia is unusual, and it may indicate a dysfunction of the reticuloendothelial system.[3]

In the past 3 years, we have investigated the significance of warm type IgG autoantibodies in blood donors discovered during compatibility testing of 69,765 RBC units. Eluates were prepared using samples from the units, and the antibodies were tested to determine their IgG subclass.

Materials and Methods

Sixty-two samples from 62 blood donors, with a mean age of 43 ± 9 years, were tested. Of these donors, 42 were males and 20 were females. All of the donors responded to a request for a further sample. A complete blood count (CBC), a reticulocyte count, and liver function tests (bilirubin [total, direct, and indirect] and transaminases [serum glutamic oxalacedic and serum glutamic pyruvic]) were performed to exclude individuals with autoimmune hemolytic anemia, as well as individuals with positive VDRL tests and viral antibodies. The following serologic tests were performed: DATs were performed by the classic tube technique using polyspecific antiglobulin serum (anti-IgG/-C3d, Ortho-Clinical Diagnostics, Raritan, NJ). In this technique, RBCs were washed × 3 using NaCl 0.9% and resuspended to a 3–5% saline suspension. Two drops of polyspecific antihuman globulin were added to 1 drop of the washed cells. The contents of the tube were mixed, centrifuged for 1 minute at 1500 rpm, and examined macroscopically for agglutination. All positive results were further tested with monospecific anti-IgG (Ortho-Clinical Diagnostics) and anti-IgA, -IgM, -C3, and -C4 (DiaMed AG, Scheitz, Switzerland). The agglutination reactions were considered as strongly positive (4+ and 3+), moderately positive (2+ and 1+), and weakly positive (w+).[4]

Gel tests, as well as tube tests, were used in parallel. Gel tests were performed according to the manufacturer's instructions for the antiglobulin card sera: IgG, IgA, IgM, and C3. All materials and reagents were obtained from DiaMed AG. For the polyspecific DAT, 50 µL of a 0.8% RBC suspension in LISS (ID-Diluent 2) was added to the top of each microtube in a LISS/Coombs ID card. The cards were centrifuged at

910 rpm for 10 minutes, using the ID-Centrifuge 24S. All positive results (presence of agglutinated RBCs in the gel matrix) were re-examined using rabbit monospecific anti-IgG, -IgA, -IgM, and -C3d cards. Each negative reaction appeared as a discrete cell button at the base of the column (DiaMed AG).

Crossmatches were performed using the LISS antiglobulin technique and ether eluates were prepared from the DAT-positive samples. The eluates were tested with a standard antibody identification panel (DiaMed AG) using polyethylene glycol (PEG 20% in PBS) or albumin 30% techniques. Polyspecific antiglobulin reagent (Ortho-Clinical Diagnostics) was used. The subclass of the IgG antibodies in the eluates was determined by adding anti-IgG1, -IgG2, -IgG3, and -IgG4 subclass antisera to the sensitized RBCs (CLB, Amsterdam, Netherlands).

Titration studies were performed by the tube test as follows: dilutions of the corresponding sera were added to one drop of 2–4% RBCs from the DAT-positive samples. The tubes were centrifuged for 1 minute at 1500 rpm and then examined for agglutination. Additional serologic tests that were performed on the DAT-positive samples included: VDRL tests for syphilis (Latex Pasteur), HIV1+2 (Abbot, Chicago, IL, AxSYM), HBsAg (Abbot, AxSYM), HTLV1/2 and HCV (ELISA, Ortho-Clinical Diagnostics). Tests for anticardiolipin antibodies (ACA) and lupus anticoagulants (LA) were performed by using ELISA (Fresenious GULL Diagnostics, Gull-Meridian Laboratory, Cincinnati, OH; and Stago Laboratory, Asnieres, France, respectively). Screening for LA included the activated partial thromboplastin time, dilute Russel's viper venom time, and kaolin clotting time. When the initial screening tests demonstrated prolonged coagulation times due to the presence of an inhibitor, confirmation that the inhibitory activity was directed against phospholipid-containing complexes was mandatory.[5] Blood samples were collected into 0.11M citrate (9 parts blood to 1 part citrate),[6] and the plasma was separated after double centrifugation.[7]

Results

Fifty-three DAT-positive samples from 62 blood donors (42 males and 20 females) were studied over the last 3 years. The samples were detected during routine compatibility testing (crossmatching) of 69,765 RBC units. Twenty-three of 53 samples reacted strongly in the IgG gel test (43.3%), 19 reacted moderately (35.8%), and 11 reacted weakly (20.7%). Twelve of the samples also reacted in the C3d gel test (22.7%). The tests with anti-IgM and -IgA were negative. Twenty-five of the 53 samples produced positive eluates while 28 produced nonreactive eluates (Table 1).

Table 1. Reactivity of samples in gel versus tube tests and elution results

Methods	Strongly positive	Moderately positive	Weakly positive
Gel test positive	23	19	11
Tube test positive	12/23	7/19	6/11
Eluate positive	12/23	7/19	6/11
Eluate negative	11/23	12/19	5/11

When the samples that reacted in the gel test were tested by IgG tube test, 12 of 23 reacted strongly (52.1%), 7 of 19 reacted moderately (36.8%), and 6 of 12 reacted weakly (54.5%) (Table 2). Six of 11 samples that reacted strongly in the IgG gel test, but produced nonreactive eluates, also reacted by the IgG tube test. Similarly, 6 of 12 samples that reacted moderately in the IgG gel test, but produced nonreactive eluates, reacted in the IgG tube test. Finally, three of five samples that reacted weakly in the IgG gel test, but produced nonreactive eluates, reacted in the IgG tube test (Table 2).

Table 2. Reactivity of gel positive samples by IgG tube test and elution results

Samples	Strongly positive	Moderately positive	Weakly positive	Total
Tube pos Eluate pos	6	1	3	10
Tube pos Eluate neg	6	6	3	15
Tube neg Eluate pos	6	6	3	15
Tube neg Eluate neg	5	6	2	13
Total	23	19	11	53

Most of the autoantibodies had titers from 4 to 8 in the tube test, and only two samples had titers of 32. Fourteen out of 25 autoantibodies (56%) produced reactive eluates that contained IgG1, one contained IgG1+IgG2, one contained IgG1+IgG4, and two contained IgG1+IgG3 weak. Four of 25 eluates were *strongly* reactive, 18 were *moderately* reactive, and 3 were *weakly* reactive. No specificity of the autoantibodies was found (broad specificity).

The DAT-positive blood donors who produced reactive eluates, 8 females and 17 males with a mean age of 42 years old (range: 28 to 60 years old), were followed for an average of 38.5 months (median follow-up 42 months). The frequency of DAT-positive samples

found by crossmatching 69,765 samples was 25, or approximately 1/3000.

Two donors with positive DATs, a woman 44 years old and a man 45 years old, were found to have low-titered ACA IgG antibodies (titers: 12 and 15 U/mL, respectively). RBCs from these two donors produced reactive eluates with titers of 8 and 16, respectively. The eluates from both of these donors contained IgG1. They have been tested every 6 months for the past 24 months, and there has been no apparent cause for these findings. None of the donors has shown any signs of hemolysis. Liver function tests, complete blood counts, and reticulocyte counts were within the normal ranges. Autoantibody tests for collagen vascular disease were normal. Viral screening tests were negative.

Discussion

The reported incidence of positive DATs among blood donors has been estimated as 1 in 14,000.[1,2] A much higher frequency of 1 in 1000 was reported by Alan and Garratty[8]; however, the discrepancy may be related to the fact that over 90 percent of the reactions were only ≤ 1+. Twenty of 22 samples were coated with IgG1, and the number of IgG1 molecules per RBC varied from 110 to 950. The remaining two samples were coated with IgG4. In another series of ten subjects, five had IgG1 only, three had IgG4, one had IgG2, and one had IgG1+IgG3. The presence of IgG3 on the RBCs of a patient without hemolytic anemia is unusual, and it may indicate dysfunction of the reticuloendothelial system.[3] A strong positive correlation with increasing age was noted in a study of hospitalized patients with positive DATs.[2] Only 1 out of 65 went on to develop autoimmune hemolytic anemia. The RBCs may be agglutinated by anti-complement (anti-C3d) or anti-IgG. The frequency of finding both IgG and complement on RBCs varies widely in different series, i.e., 15%[2], 49%[8], and 70%.[1]

Another study demonstrated that HLA-DQ6 has a negative association with a positive DAT result in patients with evidence for hemolysis, and it may be a resistance antigen for clinically relevant RBC autoantibodies.[9] The frequency of HLA-DQ6 was higher in asymptomatic DAT-positive blood donors (8.38%) than in DAT-positive hospitalized patients, 96 percent of whom had evidence of clinical hemolysis. Antiphospholipid antibodies may also be a coincidental cause of positive DATs in healthy blood donors with false positive VDRL tests.[10]

This study found 8 female and 17 male donors with positive DATs, which were confirmed by elution studies. Thus, the frequency of DAT-positive donors was one in 2790 collected blood units, i.e. ~1 in 3000. The 25 DAT-positive donors produced reactive eluates, confirming their studies. The titers of their antibodies were low (from weak to 8), and only two samples showed a titer of 32. Fourteen out of 25 positive samples (56%) demonstrated IgG1 autoantibodies, and four samples demonstrated mixed types (one of IgG1+IgG2, one of IgG1+IgG4, and two of IgG1+IgG3 weak). Two donors with low-titered ACA antibodies have been tested every 6 months. None of the donors in this study has developed autoimmune hemolytic anemia or needed therapy.

We believe that the high incidence of positive DATs among blood donors in this study can be attributed to the high sensitivity of the new methods, especially the gel technique,[11] in detecting warm IgG autoantibodies. We recommend confirming positive DAT results by the tube test DAT as well as by performing an elution.[12] Positive DATs seem to exist without symptoms for years in some people, but in others they have been associated with viral infections and immune disorders. ACA and LA antibodies have also been reported as coincidental findings in apparently healthy people.[13,14] Moreover, units from DAT-positive donors are a real nuisance in compatibility testing, especially in massive transfusions or emergency conditions. Also, units from DAT-positive blood donors are a major problem when the units are of a rare blood group. We recommend informing blood donors who have positive DATs about the laboratory finding, especially when antiphospholipid antibodies are present. In conclusion, we believe the high incidence of positive DATs among blood donors needs further investigation.

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A Reviewer's Comments:

Because the incidence of DAT-positive blood donations in this study was 25 of 69,763 samples found during crossmatching (~ 1 in 3000), the magnitude of the problem seems small; however, the authors were concerned that these units posed a problem in providing compatible units in emergency situations. Such units were regularly detected in their laboratory during compatibility testing; however, many laboratories routinely use the immediate-spin crossmatch or the electronic crossmatch, which would not detect donors with a positive DAT. A positive DAT may be age-related and benign, but it may also be symptomatic of autoimmune disease. This study presents a thought-provoking dilemma. Should we be concerned about detecting donors with a positive DAT?