Hemolytic transfusion reactions due to anti-e+f detectable only by nonstandard serologic techniques

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A patient was transfused with a total of 14 units of red blood cells (RBCs) over 33 days (January 14 to February 15) at two hospitals. Febrile transfusion reactions were noted on three occasions, and hemoglobinuria was seen twice. Alloantibodies were not detected in a sample dated February 14, following a transfusion reaction, and this sample was referred to the North London Blood Transfusion Centre. Further samples were also obtained from before and after all transfusions at both hospitals. The patient's RBCs typed as A, D+, probable Rh phenotype (cDE/cDE). The direct antiglobulin test was negative, and serum samples following the second transfusion were red/brown in color. Serologic investigations were inconclusive on all samples taken until February 13 (after the fourth transfusion). At this time, a weak anti-e reacting by manual polybrene technique and an anti-e+f reacting by two-stage papain technique were detected. The serum also contained potent HLA antibodies. The patient subsequently received leukocyte-depleted group A, cDE/cDE RBCs without any untoward effect. This case demonstrates the importance of a complete transfusion history and emphasizes that alloantibodies detectable only by nonstandard techniques can be clinically significant. Immunohematology 1994;10:120–123.

It is now commonly accepted that the use of enzyme techniques to detect red blood cell (RBC) antibodies in pretransfusion testing is unnecessary; the indirect antiglobulin technique (IAT) is accepted as the only method required. The American Association of Blood Banks (AABB) Standards do not require or recommend the use of enzyme techniques. In the 9th edition of Blood Transfusion in Clinical Medicine, the authors changed their view regarding the use of enzymes, although they still advise a second method in addition to the IAT for antibody detection. However, in the United Kingdom, the British Committee for Standardisation in Haematology (BCSH) guidelines still recommend the enzyme technique, as a supplement to the mandatory IAT. Unfortunately, the use of enzymes can give falsely positive reactions that may cause delay in supplying blood for patients and/or postponing operations. Yet the majority of "enzyme-only" antibodies are of no clinical significance, as Issitt et al. and others have conclusively demonstrated.

The term "enzyme-only" antibody refers to an antibody detected by an enzyme method and not by the standard saline IAT. However, these antibodies can on occasion be detected without an enzyme present, i.e., by using either a low-ionic strength saline (LISS) or a polyethylene glycol (PEG) IAT, a manual low-ionic strength polybrene (LIP) method, or a column agglutination antoglobulin technique. Thus, under those circumstances, such a term as "enzyme-only" is often misleading.

Enzyme methods are most useful in antibody identification or investigation of delayed transfusion reactions, when antibodies are not detected by standard serologic techniques. This case describes the presence of a potent, hemolytic anti-e+f that caused transfusion reactions on at least three separate occasions and was detectable only by a two-stage papain technique.

Case Report
A 32-year-old patient receiving radiotherapy for carcinoma of the cervix required blood transfusions. During January and February she received six transfusions at two hospitals, with a total of 14 units of RBCs being given over a period of 33 days (January 14 to February 15). Febrile transfusion reactions were noted on at least three occasions, and units transfused on February 9 and 13 were followed by hemoglobinuria. No alloantibodies had been detected by saline 37°C, two-stage papain, or LISS-IAT, and the direct antiglobulin test (DAT) was negative. A sample taken on February 14, after the transfusion of 200 mL RBCs, followed again by hemoglobinuria, was referred to the North London Blood Transfusion Centre (NLBTC) Reference Laboratory for investigation.

Materials and Methods
The patient's RBCs from the February 14 sample were typed for ABO and D, and a DAT was performed using a comprehensive panel of polyspecific anti-human
globulin (AHG), anti-C\textsubscript{3}c, -C\textsubscript{3}d, and anti-IgA reagents, according to the manufacturer’s instructions (BPL-D, Elstree, UK, and Biotest, Frankfurt, Germany). Typings for C, c, E, and e were determined using in-house reagents by a low-ionic strength polybrene (LIP) technique.\textsuperscript{8} A concentrated chloroform eluate was prepared using half as much 6-percent bovine serum albumin (BSA) (diluted from 20\% BSA [BPL-D]) than that described.\textsuperscript{9} The eluate was tested against selected in-house panel RBCs by a normal saline IAT using both polyspecific AHG and anti-IgA (BPL-D and Biotest).

Löw’s activated papain (1\%) was prepared in-house and stored in 0.5 mL aliquots at <-20\°C. Prior to use, 1 volume of freshly thawed 1-percent papain was diluted with 9 volumes of phosphate-buffered saline (pH 7.3). One volume of washed, packed RBCs was mixed with 4 volumes of 0.1-percent papain in glass tubes and incubated for 12 minutes at 37\°C. The RBCs were then washed twice in saline and resuspended to 2 percent in saline. For testing, 1 volume of pretreated RBCs was mixed with 2 volumes of patient’s serum in glass tubes and incubated at 37\°C for 20 minutes. Tubes were centrifuged and the contents inspected for lysis and examined for agglutination.

The patient’s serum was screened for RBC alloantibodies in normal saline at 20\°C and at 37\°C using an in-house red cell panel. A saline-IAT using four volumes of patient’s serum to one volume of a 3-percent RBC suspension in saline, a LIS-IAT, polyethylene glycol (PEG)-IAT, two-stage papain, and papain-IAT were also performed as described in the Technical Manual of the AABB.\textsuperscript{8} An EDTA two-stage antiglobulin test was performed according to Issitt.\textsuperscript{9} To eliminate possible antibody prozone effect, titration studies of the patient’s serum by saline-IAT were performed using 1-percent BSA (diluted from 20\% BSA [BPL-D]). LIP and LIP-IAT were also used according to the method of Malde et al.\textsuperscript{10} The patient’s serum was screened for the presence of HLA antibodies by the lymphocytotoxicity test.\textsuperscript{11}

**Results**

The first sample received by the laboratory had been collected February 14. The patient’s RBCs typed as A, D\textsuperscript{+} (most probable Rh phenotype cDE/cDE), with a negative DAT. The concentrated eluate failed to show the presence of IgG or IgA RBC antibodies on the RBCs.

Investigation of this sample revealed an apparent autoantibody reactive by two-stage papain (probably due to recent transfusions) and a weak alloanti-e by LIP only. No other RBC antibodies were detected by any other serologic technique used, including all IAT tests. The serum contained potent multispecific HLA antibodies, reacting with 100 percent of a typed lymphocyte panel, to a dilution of 1:1,024.

Figure 1 summarizes transfusions, hemoglobin (Hb), and total bilirubin levels. Records and data from both
hospitals revealed that febrile transfusion reactions had occurred on at least three occasions. Following two of the transfusions (February 9 and February 13), hemoglobinuria had been noted (suggesting hemolytic transfusion reactions [HTRs]). Serum from samples, obtained both before and after all previous transfusions at the two hospitals, showed some to be grossly hemolyzed, as seen in Figure 2.

Fig. 2. A collection of serum samples obtained during the transfusion period.

Nine serum samples dated from January 13 to February 18 were retested. Samples dated February 13 and February 18 conclusively revealed the presence of anti-e+f using a two-stage papain technique. Anti-e was the predominant specificity in LIP in the same two samples (Table 1). Samples obtained prior to February 13 showed only variable, nonspecific reactivity. No atypical RBC antibodies were detected in any other medium or by any antiglobulin test. The DAT was negative on the samples dated February 15 and 18.

The patient subsequently received four units of white blood cell filtered group A, D+, cDE/cDE RBCs via a leukocyte-depletion filter, and her Hb level rose from 7.7 to 11.1 g/dL (Figure 1).

Discussion

There is no single in vitro serologic test that detects all clinically significant antibodies in all patients. Even with a battery of sensitive techniques that do not detect antibodies in vitro, some transfused RBCs have shortened in vivo survivals and even hemolysis. On occasion, the causative antibodies of these HTRs may be deduced only from the patient's red cell phenotype.

Baldwin et al. describe a patient who experienced a clinically severe HTR, but only anti-Bga could be found in her serum. The patient typed as cDE/cDE, and 51Cr survival studies showed that 92 percent of a "test dose" of e-positive RBCs was cleared in 24 hours, while e-negative RBCs survived normally. Repeat 51Cr survival studies on a subsequent occasion confirmed the persistence of the serologically undetectable anti-e; the patient was successfully transfused with cDE/cDE blood.

A hemolytic anti-e was described by Snyder et al. that was serologically undetectable by enzyme techniques and IAT. Microbiological studies were negative, and glucose-6-phosphate dehydrogenase (G6PD), paroxysmal cold hemoglobinuria (PCH), and drug-related antibodies were all excluded as the cause of the HTR. The serum was tested by an automated polybrene technique, and anti-e was detected. Five and a half months following the transfusion reaction, the antibody was no longer detectable.

Five cases of anti-C causing delayed HTRs were reported by Pickles et al. In each of their cases, the antibody was only of low titer and best detected by enzyme methods, even after the hemolytic episodes. In one patient, the antibody could be detected only by enzymes (negative reactions by IAT).

Although it seems remarkable that antibodies that have such low titer or that are serologically undetectable can produce such severe hemolytic events, Fudenberg and Allen have noted that the in vivo effects of various antibodies may bear no relationship to their in vitro activity.

With such problems in compatibility testing, it may
seem more appropriate to use serologic methods of greater sensitivity. However, in doing so, one runs the risk of detecting clinically insignificant antibodies, such as anti-Bg\textsuperscript{a}, -Ch\textsuperscript{a}, -Rh\textsuperscript{a}, -Yk\textsuperscript{a}, and others. This would entail more time and effort in the blood bank with no increase in patient safety. Such sensitive techniques (e.g., PEG) did not detect the anti-e in this present case. An in vitro test that more closely follows in vivo action is needed, such as antibody-dependent cell-mediated cytotoxicity (ADCC) or macrophage assays.\textsuperscript{16}

Due to the clinical condition of the patient, we were unable to perform in vivo studies using RBCs that were e-positive and/or e-negative, filtered and/or unfiltered. However, it seems highly likely that the hemolysis was due to the alloanti-e, since she showed the expected increase in hemoglobin and no untoward effect from the transfusion of four units of filtered cDE/cDE RBCs.

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**Conclusion**

This case demonstrates the importance of obtaining a complete transfusion history from patients requiring blood transfusion. Hospital staff should be suspicious of a transfusion reaction where the patient’s serum is red-brown in color, even if the antibody screen yields negative results, and the case should be fully investigated before the patient receives further blood transfusion. As with previously reported cases, weakly detectable, enzyme-only antibodies have the potential to cause HTR. Although the use of enzyme-treated RBCs is not standard hospital technique, it can be useful when investigating a suspected transfusion reaction.

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**References**


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