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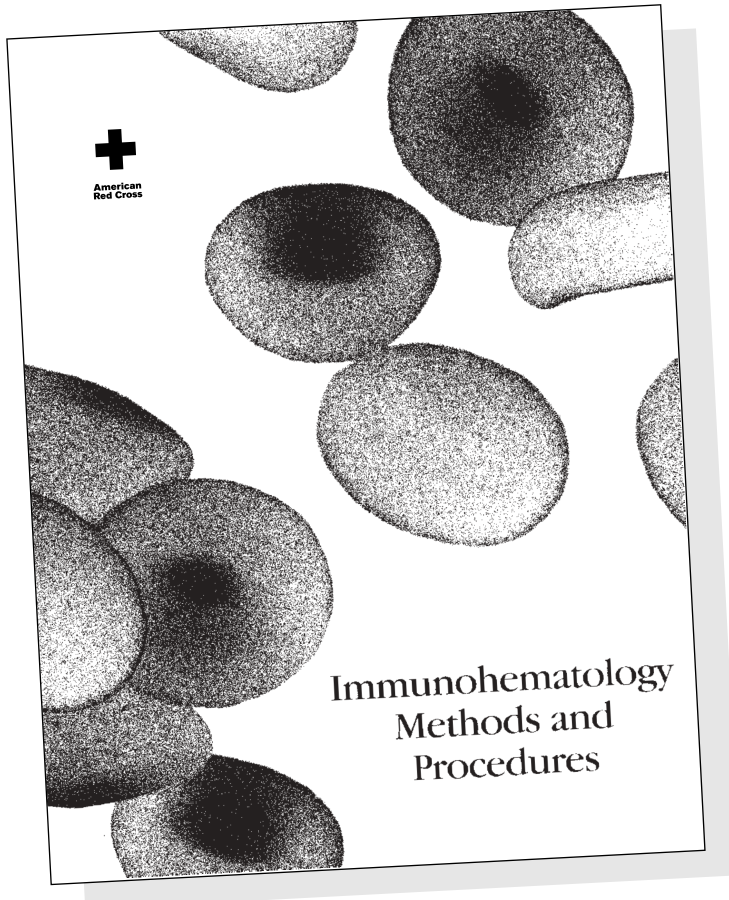

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Practical aspects of investigating drug-induced immune hemolytic anemia due to cefotetan or ceftriaxone—a case study approach

P.A. Arndt

In the 1970s, the most common causes of drug-induced immune hemolytic anemia were methyldopa and penicillin. Since 1990, the most common causes of drug-induced immune hemolytic anemia have been the second- and third-generation cephalosporins, cefotetan and ceftriaxone. Three case histories illustrate the common findings in the serologic investigation of immune hemolytic anemias due to these two drugs. ***Immunohematology* 2002;18:27–32.**

Key Words: drug-induced immune hemolytic anemia, cefotetan, ceftriaxone, case studies

Presently, most drug-induced immune hemolytic anemias (IHAs) that our laboratory investigates are due to cefotetan, a second-generation cephalosporin, or ceftriaxone, a third-generation cephalosporin. The first case of ceftriaxone-induced IHA was referred to our laboratory in November 1987[1]; since then we have identified nine more cases (40% of which were fatal). The first case of cefotetan-induced IHA was referred to our laboratory in March 1990[2]; since then we have investigated 66 more cases (16% were fatal). In contrast, since November 1987, we've investigated only seven other cases of drug-induced IHA, one each due to cefotaxime,[3] mefloquine,[4] ticarcillin,[5] or tolmetin, and three due to piperacillin.[6]

The detailed serology of our first eight ceftriaxone and 43 cefotetan cases was published in 1999.[7] The following three case studies are representative of the types of drug-induced IHA workups that we currently see in our laboratory. They illustrate some important points about investigating IHA due to cefotetan or ceftriaxone.

CASE 1

The patient was a 31-year-old woman who delivered her third child by cesarean section on March 4. She was discharged from the hospital on March 7 with a hemoglobin of 8.9 g/dL, but was readmitted on March 10 with hemolytic anemia (hemoglobin 8.7 g/dL, reticulocytes 10.5%, total bilirubin 3.3 mg/dL, LDH 503 U/L, haptoglobin < 6 mg/dL) and a 3+ positive direct antiglobulin test (DAT) with anti-IgG. Her serum contained anti-C and -e that had been previously identified and she had a history of a previous hemolytic anemia. On March 13 the hemoglobin decreased further to 6.1 g/dL and the patient was transfused with two units of red blood cells (RBCs).

The hospital blood bank technologists suspected that this patient had an antibody to cefotetan (Cefotan, Zeneca Pharmaceuticals, Wilmington, DE), as she had received two doses of that drug at the time of her surgery. These blood bank technologists had seen a previous patient with IHA due to cefotetan just 2 months earlier, so they were aware of these types of cases. The blood bank still had samples from the time of this patient's surgery. The DAT was negative the day before surgery, before she received her first dose of cefotetan. It was 2+ the day after the surgery, after she had received her second dose.

The hospital technologists checked into the patient's previous hemolytic anemia history and found that 3 years earlier, she had a cesarean section when delivering her second child and she received one dose of cefotetan at that time. Thirteen days after that surgery she was readmitted with hemolytic anemia (hemoglobin 5 g/dL, reticulocytes 15%, haptoglobin < 6 mg/dL) and she received four units of RBCs. In retrospect, the cause of this previous hemolytic anemia could also have been an antibody to cefotetan.

Further studies with this patient's current sample showed the presence of IgG (4+), C3 (2+), and IgA (3+)

on her RBCs. We have found RBCs from patients with cefotetan-induced IHA to be coated with IgG 100 percent of the time, C3 86 percent of the time, IgA 44 percent of the time, and IgM 7 percent of the time.[7]

Cefotetan-treated RBCs were prepared as previously described[7,8]: a 40 mg/mL solution of cefotetan in pH 7.3 phosphate buffered saline (PBS) was incubated with one-tenth volume of packed, fresh group O, C–e– RBCs for 1 hour at 37°C and then washed × 4. We would advise against using 6% albumin to prepare the cefotetan solution, as has been suggested for some other drugs, as cephalosporins bond efficiently to albumin. When testing drug-treated RBCs, controls are important. A positive control (i.e., a sample from a previous patient with the same drug antibody) will show that the treated RBCs are coated with the drug in question. Without this control, a negative result with the patient's sample is difficult to interpret: is drug antibody not present or are the RBCs not coated with drug? A negative control is also important. For many drugs, a pool of normal "inert" serum can be used. But for some drugs, like cefotetan or cephalothin (Keflin), that cause nonimmunologic adsorption of serum proteins onto drug-coated RBCs,[9–12] the normal serum control will be reactive. This can be overcome when testing cephalothin-treated RBCs, by diluting the normal serum and the patient's serum 1 in 20 (in PBS) to reduce the serum protein level before testing. Some normal sera diluted 1 in 20 are still reactive with cefotetan-treated RBCs (unpublished results), so a higher dilution of serum is recommended when testing cefotetan-treated RBCs (e.g., 1 in 100). *Note:* This low-titer reactivity of normal sera with cefotetan-treated RBCs has not been associated with hemolytic anemia, and in all cases of drug-induced IHA due to cefotetan that we have seen, the antibody titer has been greater than 100. Eluates can be tested without dilution as they have a low protein content. For drugs that are known to cause nonimmunologic protein adsorption, PBS can be used as a negative control.

The patient's March 3 (presurgery, predrug) serum sample was shown to contain anti-cefotetan that reacted with cefotetan-treated RBCs to a titer of 512 by antiglobulin test (untreated RBCs tested in parallel were nonreactive). The presence of preformed anti-cefotetan in this sample thus confirmed that the hemolytic anemia seen 3 years previously was likely due to cefotetan. The patient's March 10 (postdrug) serum sample reacted more strongly: the anti-cefotetan hemolyzed, agglutinated, and sensitized cefotetan-treated RBCs to titers of 128, 1024, and 128,000, respectively. The March 10 serum also reacted weakly with untreated RBCs by antiglobulin test. The presence of an autoantibody, i.e., drug-independent antibody, is not an uncommon finding in cases of IHA due to cefotetan. We found autoantibodies in about one-third of the patients with cefotetan-induced IHA that we studied (and up to 44%, if PEG was used in the test system).[7]

An acid eluate prepared from the patient's RBCs reacted with cefotetan-treated RBCs both when anti-IgG (American Red Cross, Washington, DC) and when anti-IgA (Tago, Burlingame, CA) was used, indicating that the anti-cefotetan had both IgG and IgA components. Anti-IgA is not routinely used to test eluates, but was used in this case to determine if the IgA coating on the patient's RBCs had anti-cefotetan specificity.

When eluates are tested against cefotetan-treated RBCs it is important to always test the last-wash supernatant control in parallel. Many times this last-wash control will react with cefotetan-treated RBCs (we found 74% to do so, 57% of these reacted ≥ 1+).[7,13] Although this reactivity probably relates to the fact that serum cefotetan antibodies typically have very high titers (range 4000 to 262,000; median = 20,000), we found no direct relationship between the serum anti-cefotetan titer and the presence of a reactive last wash.[13] There was a relationship seen between the presence of a reactive last wash and the strength of the patient's DAT and/or the presence of autoantibody in the patient's serum or eluate.[13] Although increasing the number of times the patient's RBCs are washed before eluate preparation is not always helpful, the type of wash solution that is used can be important. In general, we have found 4°C low-ionic-strength saline (LISS; Löw and Messeter formulation)[14] to be better than PBS as a wash solution when performing acid eluates using commercial kits (we stopped using the commercial kit wash solution because of the problem of falsely positive *eluates* in the presence of high-titer serum antibodies).[15] When LISS was used as a wash solution in cases of cefotetan-induced IHA, less strongly reactive last washes were seen.[13] This may be because low-ionic solutions help keep more low-affinity antibodies on the RBCs during the washes.

Anti-cefotetan will also react when the patient's serum is tested by the "immune complex" method.[7] Briefly, 2 drops of the patient's serum were incubated without or with 2 drops of fresh normal serum (as a source of complement) + 2 drops of a 1 mg/mL solution of drug + 1 drop of C–e– untreated or enzyme-treated RBCs for 1 to 2 hours at 37°C. Tests were examined for hemolysis and agglutination and the antiglobulin test was performed. This patient's March 10 serum reacted to a titer of 512 (antiglobulin test) versus untreated RBCs in the presence of cefotetan; enzyme-treated RBCs were strongly agglutinated; no hemolysis of test RBCs was observed.

In conclusion, this patient had a high-titer anti-cefotetan present in her serum, and her RBCs were also coated with anti-cefotetan. This antibody most likely caused the current hemolytic episode in addition to the hemolysis noted 3 years previously. This patient should be warned not to receive cefotetan again, as that may lead to a fatal hemolytic anemia.[16]

Some important points about cefotetan-induced IHA

- Cefotetan is a commonly used antibiotic, especially prophylactically with surgeries, e.g., cesarean sections.
- The hemolytic anemia usually becomes clinically apparent about 1 to 2 weeks after receiving cefotetan. Unfortunately, patients sometimes get more cefotetan at readmission (e.g., if an infection is suspected).
- A single dose of cefotetan can result in dramatic hemolytic anemia. In some cases, the hemolysis may take several weeks to subside.
- The DAT is positive; this can range from strongly (4+) to only weakly positive.
- Autoantibody (drug-independent antibody) may be present in the patient's serum and/or eluate. Thus, this drug-induced IHA can be confused with warm autoimmune hemolytic anemia, or if the patient was transfused (e.g., during surgery when cefotetan was given), a delayed hemolytic transfusion reaction may initially be suspected.
- The serum antibody (anti-cefotetan) typically reacts to a very high titer with, and may completely hemolyze, cefotetan-treated RBCs.
- The serum antibody (anti-cefotetan) usually also reacts by the "immune complex" method, but more weakly.
- Eluates prepared from the patient's DAT-positive RBCs will react strongly with cefotetan-treated RBCs.
- In a large percentage of cases, the last-wash eluate control will also react with cefotetan-treated RBCs, although usually weakly so. LISS appears to be a better wash solution than PBS for trying to reduce this problem.

CASE 2

The patient was a 76-year-old male who was admitted with a diagnosis of pneumonia. On day 1, his hemoglobin was 11.8 g/dL, creatinine 0.4 mg/dL, and serum and urine were clear. He was started on the antibiotic ceftriaxone (Rocephin; Hoffman-LaRoche, Nutley, NJ). On day 2, his creatinine was 1.0 mg/dL and hemoglobinemia was noted. On day 3, his hemoglobin/hematocrit were 8.8 g/dL/23%, total bilirubin was 1.5 mg/dL and hemoglobinemia, hemoglobinuria, and oliguria were noted. The ceftriaxone was stopped.

On day 4, his hematocrit was 21.2%, LDH 2858 U/L, serum hemoglobin 92.9 mg/dL, haptoglobin 16 mg/dL, and creatinine 4.3 mg/dL. He was transfused with two units of RBCs. Over the next 4 days, plasmapheresis was performed three times. The patient was discharged on day 11 with a creatinine of 8.9 mg/dL and no recovery of his renal function.

The patient's DAT results were negative on day 1 (before drug administration) 2½+ with anti-C3 only on day 2, and then 1+ with anti-IgG and 3+ with anti-C3 on days 3 and 4. This patient's hemolytic anemia was suspected to be due to anti-ceftriaxone. He had a history of multiple previous ceftriaxone treatments. Typically, patients with IHA due to ceftriaxone have received multiple doses of the drug.[17] In some cases, patients have had previously unrecognized hemolytic episodes.

All of the ceftriaxone antibodies that we have worked with and those that have been reported have only reacted by the "immune complex" method (i.e., patient's serum + drug + RBCs). When this patient's sera were tested by the "immune complex" method, e.g., in the presence of ceftriaxone (1 mg/mL) against untreated RBCs, agglutination was noted (titers = 4 to 16); the antiglobulin test was negative to only very weakly positive. Controls of patient's sera + PBS instead of drug were nonreactive, thus this patient's serum contained anti-ceftriaxone.

In other cases, we have seen dramatic differences in reactivity when testing enzyme-treated RBCs by the "immune complex" method.[7] For example, one patient's anti-ceftriaxone reacted to titers of 4/0 (agglutination/antiglobulin test) against untreated RBCs but reacted to titers of 256/1024 against enzyme-treated RBCs. Unfortunately, titrations of the sera from case 2 against enzyme-treated RBCs were not performed. The agglutinin in case 2 was inhibited by treatment with 0.01M dithiothreitol (DTT), and therefore appeared to be an IgM antibody. And, as in other cases of IHA due to ceftriaxone, the eluate was nonreactive.

Despite the fact that none of the previously identified ceftriaxone antibodies have reacted when tested against ceftriaxone-treated RBCs, if we have enough sample we sometimes attempt that method. We have tried coating RBCs with ceftriaxone dissolved in PBS (pH 7.3) or barbital buffer (pH 9.8),[8] or by a chemical-coupling method, e.g., using carbodiimide.[18] We tested this patient's sera against RBCs that had been treated with ceftriaxone in PBS or barbital buffer. Sera from day 3 and day 4 agglutinated (2+ and 1+, respectively) not only drug-treated but also untreated RBCs; serum from day 5 was nonreactive. As the patient received his last dose of ceftriaxone on day 3, we believe that the positive results seen on days 3 and 4 were due to circulating drug-antibody immune complexes[19,20] that were still present in the samples from those days (the half-life of ceftriaxone is about 9 hours in elderly subjects, and about 15 hours in patients with impaired renal function).[21] These immune complexes had cleared by day 5. If autoantibody had been present it would have still been detectable in the day 5 sample. If the anti-ceftriaxone had indeed been reacting with these ceftriaxone-treated RBCs, it should also have been detected in the day 5 sample. *Note:* Since no anti-ceftriaxone has ever been shown to react with ceftriaxone-treated RBCs, we had no positive control to prove that these treated RBCs were indeed coated with ceftriaxone.

In conclusion, this patient developed an anti-ceftriaxone that caused intravascular hemolysis and renal failure.

Some important points about ceftriaxone-induced IHA

- The patients have typically received multiple doses of ceftriaxone previously.
- In adults, the reaction tends to become apparent after the patient has received the drug for a day or two. In children, the reactions tend to be very dramatic, occurring within minutes of receiving ceftriaxone.[17]
- The DAT is positive due to C3 or C3 + IgG coating. The eluate is usually nonreactive.
- Ceftriaxone antibodies have only been demonstrated by the "immune complex" method (enzyme-treated RBCs react better than untreated RBCs); drug-treated RBCs are nonreactive. In two cases, antibody was only demonstrable in the presence of ex vivo drug (urine from patients receiving ceftriaxone).[22,23]
- Reactivity of the patient's serum against untreated RBCs without drug being present can be due to circulating drug-antibody immune complexes (if transient) or due to autoantibody (if persistent). This is true of any drug, not just ceftriaxone.

CASE 3

We received a telephone call from a pathologist at a commercial reference laboratory about a ceftriaxone antibody workup on a postsurgical patient who had a positive DAT and a hemoglobin of 5 g/dL.

The sample arrived a few days later with more information. The patient, a 59-year-old woman, had surgery a couple of weeks earlier and then developed a postoperative infection. On December 20, her hemoglobin was 10.3 g/dL and she received 1 g of ceftriaxone. On December 21, her hemoglobin had decreased to 8.2 g/dL and she received another 1 g of ceftriaxone. On December 22, her hemoglobin had decreased further to 5 g/dL and the ceftriaxone was discontinued. Her DAT was positive, her reticulocyte count was 3.7%, and she was transfused with four units of RBCs.

When the hospital blood bank was called to verify the patient's identification (she had the same name as another patient we had previously worked up with an IHA due to anti-cefotetan), we were told that the doctor remembered that this patient had received ceftriaxone with her bowel surgery a few weeks earlier. Thus, this patient's history was what we might expect with a ceftriaxone antibody, i.e., the patient had received the drug before and the reaction had taken a day or two to become apparent (as seen in adults).

The patient's DAT was positive (anti-IgG 1+, anti-C3 3+), but the "immune complex" testing in the presence of ceftriaxone was negative! Thus, this patient did not have a ceftriaxone antibody. We wondered, what if we had been given an incorrect history? What if the patient had received another drug (e.g., cefotetan) during the surgery a few weeks previously and not ceftriaxone? The time frame of hemolysis a couple of weeks after surgery is what would be expected in a case of cefotetan-induced IHA.

The patient's serum and eluate were tested against cefotetan-treated RBCs and found to contain anti-cefotetan. The undiluted serum hemolyzed cefotetan-treated RBCs and when diluted reacted to titers of 320 and 10,240 (agglutination and antiglobulin test, respectively). Untreated RBCs were nonreactive. The hospital blood bank was contacted and asked to determine if the patient had received cefotetan during the surgery. They checked the records and discovered that the patient had received 2 g of cefotetan with her surgery on December 10; she had been readmitted on December 20, exactly 10 days later. Luckily, she did not receive more cefotetan at the time of readmission.

In conclusion, this patient had an IHA due to anti-cefotetan, not anti-ceftriaxone.

An important point illustrated by this case

- A good history is important and may be difficult to obtain. When a drug-induced IHA is suspected, it is important to find out not only what drug(s) the patient is currently taking, but also what the patient received (e.g., in surgery) a few weeks back. This information often is "hidden" in the anesthesiologist's notes and can take some detective work to discover.

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References

1. Garratty G, Postoway N, Schwellenbach J, McMahill PC. A fatal case of ceftriaxone (Rocephin)-induced hemolytic anemia associated with intravascular immune hemolysis. Transfusion 1991;31:176-9.
2. Garratty G, Nance S, Lloyd M, Domen R. Fatal immune hemolytic anemia due to cefotetan. Transfusion 1992;32:269-71.
3. Shulman IA, Arndt PA, MeGehee W, Garratty G. Cefotaxime-induced immune hemolytic anemia due to antibodies reacting in vitro by more than one mechanism. Transfusion 1990;30:263-6.
4. Arndt PA, Garratty G, Maranto LS, Wohl H. Immune hemolytic anemia associated with mefloquine (letter). Transfusion 1997;37:1220-1.
5. Arndt PA, Wolf CF, Kripas CJ, Garratty G. First example of an antibody to ticarcillin; a possible cause of hemolytic anemia (abstract). Transfusion 1999;39(Suppl):47S.
6. Arndt PA, Garratty G, Hill J, Kasper M, Chandrasekaran V. Two cases of immune haemolytic anaemia associated with anti-piperacillin detected by the "immune complex" method. Vox Sang (in press).
7. Arndt PA, Leger RM, Garratty G. Serology of antibodies of second- and third-generation cephalosporins associated with immune hemolytic anemia and/or positive direct antiglobulin tests. Transfusion 1999;39:1239-46.
8. Petz LD, Garratty G. Acquired immune hemolytic anemias. New York: Churchill Livingstone, 1980.
9. Spath P, Garratty G, Petz L. Studies on the immune response to penicillin and cephalothin in humans. II. Immunohematologic reactions to cephalothin administration. J Immunol 1971;107:860-9.
10. Branch DR, Sy Siok Hian AL, Petz LD. Mechanism of nonimmunologic adsorption of proteins using cephalosporin-coated red cells (abstract). Transfusion 1984;24(Suppl):415.
11. Garratty G, Arndt P, Nance S. Nonimmunological adsorption of proteins onto red cells treated with second and third generation cephalosporins (abstract). Transfusion 1994;34 (Suppl):69S.
12. Garratty G, Arndt PA. Positive direct antiglobulin tests and haemolytic anaemia following therapy with beta-lactamase inhibitor containing drugs may be associated with nonimmunologic adsorption of protein onto red blood cells. Br J Haematol 1998;100:777-83.
13. Arndt PA, Leger RM, Garratty G. Reactivity of "last wash" elution controls in investigations of cefotetan antibodies (abstract). Transfusion 1999;39(Suppl):47S.
14. Löw B, Messeter L. Antiglobulin test in low-ionic strength salt solution for rapid antibody screening and cross-matching. Vox Sang 1974;26:53-61.
15. Leger RM, Arndt PA, Ciesielski DJ, Garratty G. False-positive eluate reactivity due to the low-ionic wash solution used with commercial acid-elution kits. Transfusion 1998;38:565-72.
16. Garratty G, Leger RM, Arndt PA. Severe immune hemolytic anemia associated with prophylactic use of cefotetan in obstetric and gynecologic procedures. Am J Obstet Gynecol 1999;181:103-4.
17. Moallem HJ, Garratty G, Wakeham M, et al. Ceftriaxone-related fatal hemolysis in an adolescent with perinatally acquired human immunodeficiency virus infection. J Pediatr 1998;133:279-81.
18. Petersen BH, Graham J. Immunologic cross-reactivity of cephalexin and penicillin. J Lab Clin Med 1974;83:860-70.
19. Garratty G, Houston M, Petz LD, Webb M. Acute immune intravascular hemolysis due to hydrochlorothiazide. Am J Clin Pathol 1981;76:73-8.

20. Shirey RS, Morton SJ, Lawton KB, et al. Fenoprofen-induced immune hemolysis. Difficulties in diagnosis and complications in compatibility testing. Am J Clin Pathol 1988;89:410-4.
21. Physicians' Desk Reference. 54th ed. Montvale, NJ: Medical Economics Company, Inc., 1990.
22. Meyer O, Hackstein H, Hoppe B, Göbel F-J, Bein G. Fatal immune haemolysis due to a degradation product of ceftriaxone. Br J Haematol 1999;105: 1084-5.
23. Seltsam A, Salama A. Ceftriaxone-induced immune haemolysis: two case reports and a concise review of the literature. Intensive Care Med 2000;26:1390-4.

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Neonatal alloimmune thrombocytopenia due to HPA-3a antibodies: a case report

A. Davoren, G. Smith, G. Lucas, S. Rodgers, P. O'Donoghue, J. Crowley, C.A. Barnes, and J. McKiernan

A healthy infant was born at term by elective cesarean section to a 32-year-old para 4, gravida 4, mother. Within 24 hours, the infant was noted to have fairly extensive bruising on the back and shoulders. A full blood count evaluation was remarkable for severe thrombocytopenia (platelet count of 29×10^9/L). Other hematologic parameters were normal. Human leukocyte antigen (HLA) class-1 antibodies but not platelet-specific antibodies were detectable in the maternal serum using a commerical antigen-capture ELISA (GTI-PakPlus kit®). Anti-HPA-3a antibodies, while weakly reactive in the monoclonal antibody immobilization of platelet antigens (MAIPA) assay in the immediate postpartum serum, were readily detectable using this assay in a sample taken 4 weeks later. Genotyping for human platelet antigens (HPA) 1–5 by the polymerase chain reaction technique with sequence-specific primers (PCR-SSP) revealed the infant's platelet genotype to be *HPA-1a/1a, 3a/3b,* while that of the mother was *HPA-1a/1a, 3b/3b,* consistent with a diagnosis of anti-HPA-3a neonatal alloimmune thrombocytopenia (NAIT). This case illustrates the increased sensitivity of the MAIPA technique for the detection of platelet-specific antibodies. We believe this to be the first serologically confirmed case of NAIT due to anti-HPA-3a to be reported in the republic of Ireland. ***Immunohematology*** **2002;18:33–36.**

Key Words: anti-HPA-3a, MAIPA, NAIT

Neonatal alloimmune thrombocytopenia (NAIT) is the result of platelet destruction by maternal IgG alloantibodies directed against antigens on fetal/neonatal platelets.[1] It occurs in approximately 1 in 1000 live births.[2,3] Clinical sequelae vary from asymptomatic thrombocytopenia in some infants to intracranial hemorrhage in the more severely affected cases.[3,4] There is a reported mortality in NAIT of up to 10 percent of affected infants, while a further 10–20 percent suffer varying degrees of neurologic impairment due to intracranial hemorrhage.[5–7]

Several human platelet antigen (HPA) systems have been identified.[8] Most are biallelic, with the high-frequency antigen being designated the "a" antigen and the low-frequency antigen, the "b" antigen. HPA-1a is the most clinically relevant platelet antigen in Caucasians, with anti-HPA-1a alloimmunization in *HPA-1b* homozygous mothers accounting for approximately 85 percent of cases of NAIT.[4] An additional 10–15 percent of cases are caused by HPA-5b antibodies.[4] NAIT due to other platelet antigen incompatibilities is relatively uncommon. We describe a case of NAIT due to maternal HPA-3a alloimmunization.

Case Report

A 32-year-old mother gave birth to her fourth child by elective cesarean section after an uncomplicated pregnancy. She had three previous healthy children and an uncomplicated obstetric and perinatal history. She had not taken any medications during her current pregnancy, had no history of blood transfusion, was immune to rubella, and was negative for hepatitis B surface antigen.

The male infant (birth weight: 3820 g) was generally healthy at birth, with Apgar scores of 9 and 10 at 1 and 5 minutes, respectively. Approximately 24 hours after delivery, the infant was noted to be irritable and physical examination revealed the presence of petechiae and bruising on the left arm and back, extending to the right shoulder region. The infant's platelet count was 29×10^9/L, hemoglobin 16.4 g/dL, activated partial thromboplastin time (APTT) 34 seconds (control 26 to 32 seconds), and international normalized ratio (INR) 1.3. Red and white blood cell counts were normal. There was no evidence of infection, malformation, hemangioma or hepatosplenomegaly. The maternal platelet count was normal and there was no familial history of bleeding disorders or NAIT. Blood cultures were negative. A clinical diagnosis of NAIT was made and the infant was observed closely in the special care baby unit, where he remained well with no extension of bruising, and his behavior was normal. Platelet transfusion was not required. At discharge on day 7, the infant's platelet count was 118 $\times 10^9$/L. At follow-up 4 weeks after discharge from the hospital, the baby was developing normally and had a normal platelet count (353×10^9/L).

Materials and Methods

A maternal serum sample, obtained after delivery, was tested for platelet-reactive antibodies, using a commercial antigen-capture enzyme-linked immunosorbent assay (ELISA) kit (GTI-PakPlus® ELISA, Quest Biomedical, Knowle, West Midlands, UK).[9] This kit was used with an IgG conjugate and in accordance with the manufacturer's instructions. The mother's serum was also referred to the International Blood Group Reference Laboratory (IBGRL), Bristol, UK, for testing by the platelet suspension immunofluorescence technique (PSIFT)[10] and by the monoclonal antibody immobilization of platelet antigens (MAIPA) assay.[11] The immunofluorescence test was assessed by the use of flow cytometry. A maternal serum sample obtained 4 weeks later for repeat investigation was referred to both the IBGRL and the Platelet Immunology Reference Laboratory, National Blood Service, Cambridge, UK. HPA genotyping was performed using the polymerase chain reaction technique with sequence-specific primers (PCR-SSP) for HPA 1–5.[12]

Results

Human leukocyte antigen (HLA) antibodies class-I, but not platelet-specific antibodies, were detectable in both the serum sample obtained after delivery and the repeat sample taken 4 weeks later, using the GTI PakPlus® kit. The patient's serum bound both IgG and IgM to the surface of platelets in the immunofluorescence test. While weakly reactive HPA-3a antibodies were identified by the MAIPA assay in the immediate postpartum serum, in addition to HLA class-I antibodies, anti-HPA-3a was readily detectable in a sample taken 4 weeks later.

The mother's platelet genotype was *HPA-1a/1a, HPA-3b/3b,* and the infant typed as *HPA-1a/1a, HPA-3a/3b,* as determined by PCR-SSP. These results were consistent with a diagnosis of NAIT due to maternal HPA-3a antibodies. Paternal platelets were unavailable for typing or crossmatch studies with maternal serum.

Discussion

NAIT occurs in approximately 1 in 1000 pregnancies.[2,3] HPA-1a antibodies account for about 85 percent of cases and anti-HPA-5b for 10–15 percent of cases.[4] NAIT resulting from alloimmunization to the HPA-3a (Bak[a]) antigen in *HPA-3b* homozygous mothers is rare (< 1% of documented cases).[4]

Of 27 cases of serologically confirmed NAIT diagnosed in Ireland between January 1992 and December 2000 (A. Davoren, in press), 25 (93%) were due to HPA-1a antibodies, one was due to HPA-5b antibodies, and one (this case) was due to HPA-3a antibodies. In the study by Mueller-Eckhardt, et al.,[4] only one out of 121 serologically confirmed cases of NAIT was due to HPA-3a antibodies.

Antibodies to the HPA-3a (Bak[a]) antigen system were originally described in 1980[13] and the antigen was localized to glycoprotein IIa. In that first reported case, the first child of a healthy mother developed severe thrombocytopenia and died of a cerebral hemorrhage on day 4 of life. Only a small number of cases have been reported since 1980 but all have been associated with severe thrombocytopenia (platelet count < 30×10^9/L).[14–20] One infant sustained an intracranial hemorrhage with residual hemiparesis, mental retardation, and epilepsy.[19] Thus, although HPA-3a would appear to be significantly less immunogenic than either HPA-1a or -5b, NAIT caused by HPA-3a antibodies is similar in its severity to anti-HPA-1a–induced disease.[20] Furthermore, the duration of thrombocytopenia associated with HPA-3a alloimmunization may be prolonged.[19]

Improvements in platelet antibody detection techniques and PCR technology have led to increased diagnosis of NAIT in recent years. However, considerable inconsistency in test results between laboratories continues to be reported for antibodies other than anti-HPA-1a.[21] The fragility of the Bak[a] epitopes has been demonstrated previously and may account for the difficulties sometimes encountered in the detection of these antibodies.[22,23]

The use of a commercial antigen-capture ELISA (GTI PakPlus®) failed to detect the HPA-3a antibody in this case. In contrast, the MAIPA assay was able to detect HPA-3a antibodies in both the immediate postpartum serum sample (weakly) and, more readily, in the sample taken 4 weeks later.

The advantages of the MAIPA assay for HPA alloantibody detection compared to the GTI kit have been previously documented.[9] In the MAIPA technique, monoclonal antibodies are used to isolate the various platelet glycoproteins from each other, thus permitting analysis of mixtures of antibodies directed against different antigens.[11]

This case highlights potential difficulties that may be encountered in the detection of platelet-specific antibodies and illustrates that more than one assay, combined with platelet genotyping, may be required for the diagnosis of NAIT. Weak or undetectable antibodies can be boosted to readily detectable levels in the weeks after delivery. Thus, it is important to obtain follow-up samples from patients where there is a strong clinical suspicion of NAIT. Accurate characterization of the responsible platelet-specific antibody is important to enable appropriate counseling of the parents regarding future pregnancies and to avoid complications if blood transfusions are required.

References

1. Kickler TS. Neonatal alloimmune thrombocytopenia. Clin Lab Med 1992;12:577-86.
2. Dreyfus M, Kaplan C, Verdy E, et al. Frequency of immune thrombocytopenia in newborns: a prospective study. Blood 1997;89: 4402-6.
3. Williamson LM, Hackett GA, Rennie JM, et al. The natural history of feto-maternal alloimmunisation to the platelet-specific antigen HPA 1a (PL^{A1}, Zw^{a}) as determined by antenatal screening. Blood 1998;92:2280-7.
4. Mueller-Eckhardt C, Kiefel V, Grubert A, et al. 348 cases of suspected neonatal alloimmune thrombocytopenia. Lancet 1989;1:363-6.
5. Spencer JA, Burrows RF. Feto-maternal alloimmune thrombocytopenia: a literature review and statistical analysis. Aust N Z J Obstet Gynaecol 2001;41: 45-55.
6. Bonacossa IA, Jocelyn LJ. Alloimmune thrombocytopenia of the newborn: neurodevelopmental sequelae. Am J Perinatol 1996;13:211-5.
7. Khouzami AN, Kickler TS, Callan NA, et al. Devastating sequelae of alloimmune thrombocytopenia: an entity that deserves more attention. J Matern-Fet Med 1996; 5:137-41.
8. Santoso S, Kiefel V. Human platelet-specific alloantigens: an update. Vox Sang 1998;74(Suppl.2): 249-53.
9. Lucas GF, Rogers SE. Evaluation of an enzyme-linked immunosorbent assay kit (GTI PakPlus) for detection of antibodies against human platelet antigens. Transfus Med 1999;9(1):63-7.
10. Von dem Borne AEGKr, van Leeuwen EF, von Riesz LE, et al. Neonatal alloimmune thrombocytopenia: detection and characterisation of the responsible antibodies by the platelet immunofluorescence test. Blood 1981;57(4):649-56.
11. Kiefel V, Santoso S, Weisheit M, Mueller-Eckhardt C. Monoclonal antibody-specific immobilisation of platelet antigens (MAIPA): a new tool for the identification of platelet specific antibodies. Blood 1987;70:1722-6.
12. Cavanagh G, Dunn AN, Chapman CE, et al. HPA genotyping by PCR sequence-specific priming (PCR-SSP): a streamlined method for rapid routine investigations. Transfus Med 1997;7:41-5.
13. von dem Borne AEGK, von Riesz E, Verheugt FWA, et al. Bak(a), a new platelet specific antigen involved in neonatal alloimmune thrombocytopenia. Vox Sang 1980;39:113-20.
14. Hidajat M, Deckx H, Van Eygen M, Logghe N, Criel A. Neonatal alloimmune thrombocytopenic purpura induced by anti-Bak(a): a case report and review of the literature. Acta Clin Belg 1989;44:377-82.
15. Boehlen F, Kaplan C, de Moorloose P. Severe neonatal alloimmune thrombocytopenia due to anti-HPA-3a. Vox Sang 1998;74:201-4.
16. Eisen M, Motum P, Gibson J, et al. Neonatal alloimmune thrombocytopenia caused by an antibody to the Bak(a) antigen. Pathology 1990;22:203-5.
17. Nathan FE, Herman JH, Keashen-Schnell M, et al. Anti-Bak(a) neonatal alloimmune thrombocytopenia: possible prevention by intravenous immunoglobulin. Pediatr Hematol Oncol 1994;11:325-9.
18. Takada H, Nakamura S, Nishiguchi T, et al. Neonatal alloimmune thrombocytopenia associated with anti-human platelet antigen-3a antibody. Acta Paediatr Jpn 1997;39:371-4.
19. Miller DT, Etzel RA, McFarland JG, Aster RH, White II GC. Prolonged neonatal alloimmune thrombocytopenic purpura associated with anti-Bak^{a}. Two cases in siblings. Am J Perinatol 1987;4:55-8.
20. Glade-Bender J, McFarland JG, Kaplan C, Porcelijn L, Bussel JB. Anti-HPA-3a induces severe neonatal alloimmune thrombocytopenia. J Pediatr 2001; 138(6):862-7.
21. Metcalfe P, Allen D, Chapman J, Ouwehand WH. Interlaboratory variation in the detection of clinically significant alloantibodies against human platelet alloantigens. Br J Haematol 1997;97:204-7.
22. Take H, Tomiyama Y, Shibata Y, et al. Demonstration of the heterogeneity of epitopes of the platelet-specific alloantigen, Bak^{a}. Br J Haematol 1990;76:395-400.
23. Minchinton RM, Dawkins B, Chynoweth L, et al. In pursuit of enigmatic platelet antibodies—anti-HPA-2b and anti-HPA-3a. Tranfus Med 1996;6:289-91.

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Anti-Mt^a associated with three cases of hemolytic disease of the newborn

C.C. Cheung, D. Challis, G. Fisher, S.J. Russell, A. Davis, H. Bruce, J. Watt, and B.H. Chong

The Mt^a antigen is a low-frequency red blood cell (RBC) surface antigen and is an established antigen of the MNSs blood group system. There has been one report of anti-Mt^a–induced hemolytic disease of the newborn (HDN) in the literature to date. We describe a family in which three children were affected by neonatal anemia. The clinical and hematologic findings were consistent with HDN, despite repeatedly negative direct antiglobulin tests (DAT) on cord RBCs. Serologic investigations showed that the mother's serum contained anti-Mt^a. The father and all three children phenotyped as Mt^a+, while the mother was Mt^a–. Adsorption and elution experiments gave results which suggested that anti-Mt^a may be implicated in recurrent HDN in this family. ***Immunohematology*** **2002;18:37–39.**

Key Words: Mt^a, MNSs blood group system, hemolytic disease of the newborn (HDN), direct antiglobulin test (DAT)

The rare Mt^a antigen was first described by Swanson and Matson in 1962.[1] In a kindred with four generations available for testing, they demonstrated that Mt^a was a red blood cell (RBC) antigen of the MNSs system, and the inheritance pattern was autosomal dominant. Subsequently, work by Konugres et al.[2] produced anti-Mt^a in rabbits. This allowed large-scale screening to be performed. Twenty-nine Mt^a+ individuals were detected among 12,914 unrelated people in Boston, and all 29 were also N+s+.[2]

There is only one report published of Mt^a-associated pathology. Field et al.,[3] in 1972, described a family in which the third child was affected by hemolytic disease of the newborn (HDN).[3] The father, the second child, and the newborn were all Mt^a+, while the mother was Mt^a–. Anti-Mt^a was eluted from the newborn's RBCs.

In the following report, we describe a family in which all three live children were affected by HDN to varying degrees. Anti-Mt^a was identified in the maternal serum, while the father and all three children were Mt^a+. Extensive investigations for causes of hemolytic anemia in the children did not establish alternative diagnoses. Circumstantial evidence, therefore, suggests that anti-Mt^a caused HDN in all three cases.

Materials and Methods

Sample collection

Whole blood was collected in EDTA, then centrifuged at 3000 rpm for 10 minutes to separate RBCs from plasma, then stored at 4°C until used.

Routine antibody screen

The Ortho Biovue™ System (Ortho-Clinical Diagnostics, Raritan, NJ) was used for routine antibody screens. The Ortho Biovue System uses cassettes containing six columns of glass beads. Each column contains anti-IgG and -C3d polyspecific anti-human globulin (rabbit and murine monoclonal) in solution. Screening by the indirect antiglobulin test (IAT) uses group O reagent RBCs supplied by CSL Ltd., Parkville VIC, Australia. Ten µL of a RBC suspension (3% to 5%) and 40 µL of patient's plasma are added to a reaction chamber above a column. The cassette is incubated at 37°C for 10 minutes (Ortho Biovue™ System heat block), then centrifuged in an Ortho Biovue™ System centrifuge. RBCs retained in or above the glass bead column is a positive result. Samples giving a positive result were tested further. A button of packed RBCs at the bottom of the column represents a negative result.

Direct antiglobulin test (DAT)

Approximately 200 µL of packed RBCs are washed × 3 in buffered normal saline (0.9%). Two drops of anti-human globulin (anti-IgG and -C3d, CSL, Ltd.) are added to the cell button. The sample is centrifuged at 3000 rpm for 15 seconds and examined both macroscopically and microscopically. If the test is negative, it is left at room temperature for 5 minutes, then recentrifuged and examined. One drop of IgG-sensitized RBCs is added to negative tests as a control for the anti-human globulin reagent.

Eluates

Elution was performed using the heat method and eluates were tested by BioVue^R IAT.

PEG indirect antiglobulin test

Polyethylene glycol (PEG)-IAT was carried out with PeG™, supplied by Gamma Biologicals, Inc., Houston, Texas, in accordance with the manufacturer's directions.

Case History

The mother was a healthy 38-year-old woman in her fifth pregnancy by the same partner. Her blood group was A, D+ with no antibody detected on routine screening using the BioVue^R(Ortho) system. Her husband was also A, D+. She had two miscarriages before her first liveborn group A, D+ child, now 10 years old. This child was born at term with severe anemia, gross hepatosplenomegaly, and hydrops, but direct antiglobulin test (DAT) negative. At birth the hemoglobin was 48 g/L, accompanied by marked polychromasia on blood film, and she required packed RBC transfusions and intensive care support. Recovery was prompt and her hemoglobin eventually increased to normal. Investigations for intrauterine infection and other possible causes of anemia yielded no definite diagnosis. There were no long-term sequelae, and subsequent development was normal.

The second child was born 3 years later. He was delivered by cesarean section at 39 weeks of gestation due to fetal distress. He was jaundiced at birth, with a low hemoglobin for his age (110 g/L) and elevated serum bilirubin level of 107 μmol/L (reference range 0–15 μmol/L). His blood group was A, D+, and the DAT was negative. He was treated with phototherapy. Exchange transfusion was not required. About 2 weeks postdelivery, he was readmitted with a hemoglobin level of 72 g/L. No hepatosplenomegaly was evident. Extensive investigations were carried out to exclude hereditary conditions such as a hemoglobinopathy, RBC enzyme, or membrane defect. No definite diagnosis was established. He recovered spontaneously without transfusion and with subsequent normalization of his hemoglobin on follow-up. His subsequent development was also normal.

The proband was the third child, born in February 2000. He had been monitored antenatally as a high-risk pregnancy, and he was delivered by cesarean section at 34 weeks of gestation for moderate polyhydramnios and mild hydrops. At birth he was in respiratory distress, pale, and jaundiced. There was moderate hepatosplenomegaly. His hemoglobin level was 108 g/L, his blood group was A, D+, and the DAT was negative. His serum bilirubin at birth was 90 μmol/L and it peaked at 162 μmol/L on day 3. A RBC exchange transfusion and intensive care support were instituted. He responded well to treatment, requiring only one exchange transfusion, and was discharged after 2 weeks. His hemoglobin on follow-up has been normal.

The newborn's blood film showed marked polychromasia and erythroblastosis. The appearance was consistent with moderate to severe hemolysis associated with HDN. Further serologic investigations were pursued in spite of the negative DAT result on his RBCs.

The mother's serum was tested against the father's RBCs. The test was positive by IAT using the BioVue^R(Ortho) system. This was further evidence for maternal RBC antibody as the cause of the clinical picture. Since the mother had no detectable antibody in her plasma on routine screening with commercial screening cells, this finding suggested an antibody to a low-frequency antigen. The case was referred to an Australian Red Cross reference laboratory.

At the reference laboratory, antibody screening of the mother's serum was repeated with commercial screening cells, using room temperature saline, papain-treated indirect antiglobulin test (papain-IAT), and polyethylene glycol indirect antiglobulin test (PEG-IAT). Again, no antibody was detected.

Further testing involved crossmatching the mother's serum with her husband's RBCs and the baby's RBCs. Both the husband's RBCs and the baby's RBCs were strongly incompatible with the mother's serum, but this was demonstrable only by the PEG-IAT method. Because they reacted in an identical pattern, this suggested that both baby and husband expressed the same low-frequency antigen. Absence of reaction by the papain method suggested the antigen was sensitive to papain. DTT-treated serum resulted in no reduction in reaction strength, indicating the antibody was not IgM and, therefore, likely to be IgG.

Based on the above tests, a literature search was performed to identify candidate low-frequency antigens.[4–6] The MNS system was the first to be closely examined, as it is known that most antigens in this system are enzyme sensitive, and there are a number of low-frequency antigens whose alloantibodies have been associated with HDN. Further testing of the mother's serum against a panel of low-frequency antigens was performed. Strong reactions were detected against four Mt^a+ RBCs by PEG-IAT.

Phenotyping of RBCs from the father and the three children with anti-Mt^a demonstrated that they were all Mt^a+, while the mother was Mt^a–. Although an eluate prepared from the neonate's cord RBCs did not show Mt^a activity, anti-Mt^a was weakly detectable in the serum from the cord blood sample. ABO, Rh, and MNSs phenotypes of the father, mother, and the three children are shown in Table 1.

Table 1. Family phenotype

Family	ABO, Rh	Rhesus*	MNSs	MT^a
Mother	A, D+	CDe/cDE	M+N+S+s+	Mt^a–
Father	A, D+	cDE/cde	M+N+S+s+	Mt^a+
1st child	A, D+	cDE/cDE	M–N+S–s+	Mt^a+
2nd child	A, D+	CDe/cDE	M–N+S–s+	Mt^a+
3rd child	A, D+	cDE/cde	M–N+S–s+	Mt^a+

*Most probable Rh phenotype

Adsorption of the mother's serum by RBCs from her second child resulted in a positive DAT. The antibody was shown to be IgG, based on the specific antiglobulin reagent. An eluate from these cells reacted against known Mt^a+ RBCs, thereby confirming the specificity of the antibody.

Discussion

In this family, three children were born with variable degrees of anemia, jaundice, and hydrops. All of them recovered after the perinatal period, maintaining normal hemoglobin levels on follow-up. The most common cause of such a clinical scenario would be HDN. Intrauterine infections were excluded after all three births. Hereditary RBC or hemoglobin defects would be unlikely given the negative investigation results at presentation, as well as subsequent normal hemoglobin levels in all three children. Given (1) that the mother's serum contained anti-Mt^a, (2) that the father and all three children are Mt^a+, and (3) the exclusion of other common causes for such a clinical presentation, the finding of anti-Mt^a is considered to be the likely cause of recurrent HDN in this family.

Under the circumstances, it was not possible to prove definitively that anti-Mt^a was the cause of HDN. Proof would have required that the neonate's RBCs be demonstrably coated with antibodies (i.e., DAT positive) and that the eluate from the neonate's cells be reactive against known Mt^a+ RBCs. In this baby's case, as was the case with his older brother, the DAT had been consistently negative, and elution was attempted but did not demonstrate anti-Mt^a.

In the current case, the sensitizing events for the mother could have been the two miscarriages before her liveborn children.

One confusing factor in the management of this case was the repeatedly negative DATs on cord cells of the affected children. In contrast, in the case described by Field et al.,[3] a strongly positive DAT was demonstrated on the baby's RBCs. This variation in clinical presentation remains unexplained, but it serves to illustrate that a negative DAT on anemic neonates may not necessarily exclude HDN.

References

1. Swanson J, Matson GA. MT^a, a "new" antigen in the MNSs system. Vox Sang 1962;7:585-90.
2. Konugres AA, Fitzgerald H, Dresser R. Distribution and development of the blood factor Mt^a. Vox Sang 1965;10:206-7.
3. Field TE, Wilson TE, Dawes BJ, Giles CM. Hemolytic disease of the newborn due to anti-Mt^a. Vox Sang 1972;22:432-7.
4. Issitt PD, Anstee DJ. Applied blood group serology. 4th ed. Durham, NC: Montgomery Scientific Publications, 1998:495-6.
5. Reid ME, Lomas-Francis C. The blood group antigen facts book. San Diego: Academic Press, 1997:50-1.
6. Daniels GF. Human blood groups. Oxford: Blackwell Science, 1995:185.

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Moderate hemolytic disease of the newborn (HDN) due to anti-Rh17 produced by a black female with an e variant phenotype

M.C. Brumit, G.E. Carnahan, J.R. Stubbs, J.R. Storry, and M.E. Reid

The Rh blood group antigen e is of high incidence and has many epitopes. Partial expression may occur, more commonly in black persons. Individuals with e variant phenotypes can make antibodies to epitopes they lack. While some of these antibodies may be specific for an antigen, e.g., hr^B, others, like anti-Rh17 (anti-Hr_o), show broader specificity, compatible only with D– – and Rh_{null} red blood cells (RBCs). Anti-Rh17 in persons of the D– – phenotype has been reported to cause mild to fatal HDN. We report an example of anti-Rh17 produced by a black female with an e variant RBC phenotype that caused moderate HDN. A panel of seven monoclonal anti-e demonstrated her RBCs carried a variant e antigen, and her genotype was *RHD, RHce* by PCR-RFLP analysis. Amniotic fluid with ΔOD_{450} values from 30 to 35 weeks' gestation predicted moderate HDN probability by the Liley method. At 38+ weeks, a viable 3165 g female infant was delivered. The infant's direct antiglobulin test was 2+ with anti-IgG. Total bilirubin rose to 14.2 mg/dL within 48 hours. Indirect bilirubin peaked at 14.7 mg/dL. The bilirubin responded to triple phototherapy. The infant was discharged on day 6. Potential for infant morbidity due to anti-Rh17-mediated HDN and the importance of specifying risks to women with this antibody if they contemplate pregnancy are discussed. ***Immunohematology* 2002:18:40–42.**

Key Words: hemolytic disease of the newborn (HDN), anti-Rh17, variant e phenotype

The Rh blood group antigen e occurs with an incidence of approximately 98 percent and consists of many epitopes.[1] Partial expression, and thus the absence of specific epitopes, can occur particularly in black persons. After transfusion or, less often, pregnancy, individuals with partial e expression can become immunized to the various epitopes of the RhCE protein that they lack.[2] Anti-Rh17 usually occurs in individuals with various deletion phenotypes such as D– –, and has been reported as a cause of mild to fatal hemolytic disease of the newborn (HDN). Pregnancies as early as the second and late as the fifth have been affected. In addition to HDN, previously reported cases describe loss of pregnancy and maternal hemorrhage. Such complications occurred in first as well as higher order gestations.[3–5] Anti-Rh17 also occurs in individuals with variant expression of the e antigen and is sometimes noted as anti-Rh17-like. We report moderate HDN due to anti-Rh17 during the second pregnancy in a woman with e variant phenotype.

Case Report

At 8 weeks' gestation, a positive antibody screen was found in a group A, D+, gravida 2, 27-year-old, black female. Her first pregnancy was unremarkable and she had no history of transfusion. An antibody investigation revealed anti-Rh17. Her Rh phenotype was D+C–E–c+e+. The initial antibody titer of 32 rose to 64 at 28 weeks and amniotic fluid ΔOD_{450} values from 30 to 35 weeks' gestation predicted moderate HDN by the Liley method. The pregnancy was otherwise uncomplicated. At 38+ weeks, a viable 3165 g female infant with Apgar scores of 8 and 9 was born by induced vaginal delivery. The infant's RBCs were group A, D+ and the direct antiglobulin test was positive (2+) with anti-IgG. While hemoglobin values remained in an acceptable range, 13 to 13.4 g/dL, total bilirubin rose from 2.6 to 14.2 mg/dL within 48 hours of delivery. Indirect bilirubin peaked at 14.7 mg/dL. After 3 days of triple phototherapy, the total bilirubin was 9.7 mg/dL. The infant was discharged on day 6.

Materials and Methods

Hemagglutination tests

Standard hemagglutination techniques were used throughout. The patient's RBCs were typed with a panel of monoclonal (Mab) anti-e (MS16, MS17, MS19, MS21, MS62, MS63, MS69; Bioscot, Ltd.), a monoclonal anti-hr^B-like (FOR 2E3), and polyclonal antibodies to high-incidence Rh antigens that included, anti-hr^S, -Rh17, -Rh29, and -Rh46. The patient's serum was tested with a panel of e variants and D– – and Rh_{null} RBCs by the gel IgG card.

DNA analysis

DNA analysis using routine polymerase chain reaction (PCR) techniques that included allele-specific PCR, multiplex PCR, and PCR-restriction fragment length polymorphism (RFLP) was used to characterize the *RHD* and *RHCE* genes of the patient.[6]

Results

Hemagglutination

The patient's RBCs typed hr^B– with Mab FOR 2E3 and were nonreactive with one example of anti-Rh17. The RBCs were strongly reactive with another example of anti-Rh17. This is not surprising since these antibodies are heterogeneous and the patient is likely to have partial expression of Rh17 (Hr_o). The RBCs were strongly reactive with one example of anti-Rh29 and with three examples of anti-Rh46. The RBCs typed hr^S+ but were less reactive than the E+e+ control RBCs, and they reacted moderately with three of seven Mab anti-e.

The patient's serum was strongly reactive with a panel of RBCs of normal Rh phenotype and with 6 RBC samples known to carry an e variant phenotype. Two examples each of D– – and Rh_{null} RBCs were compatible. These results show that the serum contained an antibody with Rh17 specificity. Collectively, our results suggest that the patient's RBCs carry an e variant phenotype in which epitopes of the Rhce protein are absent and that the patient has produced an antibody (anti-Rh17 or anti-Rh17-like) to the epitopes absent from her RBCs.

DNA analysis

The patient's genotype was *RHD*, *RHce*. The predicted phenotype from these results is D+C–E–c+e+. The patient was also homozygous for a mutation 48G>C of the *RHce* gene. While this mutation is common in black individuals with the R_o phenotype, it has also been shown to be associated with an altered e antigen in whites.[7]

Discussion

The e blood group antigen is complex, particularly when there is partial expression. In individuals with partial e phenotypes, antibodies with anti-e-like specificity have been described. The specificity of these antibodies often broadens upon repeat transfusion, or following pregnancy,[2] so that only RBCs of the D– – or Rh_{null} phenotypes are compatible. These antibodies are called anti-Hr_o (Rh17) and may have separable components when subjected to complex adsorption and elution procedures. Anti-Rh17 has caused HDN and has been associated with other maternal and fetal complications: spontaneous abortion and maternal hemorrhage have been reported as early as the first pregnancy and in higher order gestations. Infants have been successfully managed with transfusion of washed maternal RBCs and phototherapy, while maternal complications have been corrected with autologous or Rh_{null} RBC transfusions.[3–5] We report moderate HDN successfully managed with phototherapy. Three maternal RBC units were collected during the third trimester, but were not transfused.

The patient's genotype was *RHD, RHce* by DNA analysis. She was homozygous for a 48G>C mutation on the *RHce* gene, which in blacks is common and associated with the R^o haplotype. Its association with an e variant phenotype has previously been described only in whites.[7] While the serologic studies indicate this patient's RBCs carry an e variant phenotype, the exact nature of the Rhce protein and which epitopes are absent remains to be defined.

This case of moderate HDN, occurring during a second pregnancy in the presence of anti-Rh17 or anti-Rh17-like, supports the previously reported cases of severe HDN and fetal death associated with anti-Rh17. These risks should be explained to women with this antibody who contemplate multiple pregnancies. As with any antibody to a high-incidence antigen, it is helpful to test samples from the patient's siblings to find compatible blood.

Acknowledgments

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References

1. Issitt PD. An invited review: the Rh antigen e, its variants, and some closely related serological observations. Immunology 1991;7:29-36.
2. Issitt PD, Anstee DJ. The Rh blood group system. In: Applied blood group serology. Durham, NC: Montgomery Scientific Publications, 1998:315-423.
3. Dietenbeck R, Tutschek B, Stannigel H. Successful management of pregnancy and hemolytic disease of the newborn due to anti-Hr^{o} in a woman of the D– phenotype (Letter). Transfusion 1999;39:1151-2.
4. Cotorruelo C, Biondi C, Rosasco MG, et al. Deletion of E and e antigens in a pregnant woman (letter). Transfusion 1996;36:191.
5. Han KS, Kim HC, Han KS, et al. A case of fatal hemolytic disease of the newborn associated with –D–/–D– phenotype. Am J Perinatology 1997;14: 495-7.
6. Denomme GA, Rios M, Reid ME. Molecular protocols in transfusion medicine. San Diego, CA: Academic Press, 2000.
7. Westoff CM, Silberstein LE, Wylie DE, Skardahl M, Reid ME. 16 Cys encoded by the *RHce* gene is associated with altered expression of the e antigen and is frequent in the *R*^{o} haplotype. Br J Haematol 2001;113:666-71.

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MIMA-9, a valuable antibody for screening for rare donors

E. Tossas, R. Øyen, G.R. Halverson, H. Malyska, and M.E. Reid

Since monoclonal antibodies (Mabs) are potentially available in an unlimited volume, they can be used to screen numerous donor blood samples to identify antigen-negative donors. We have used a Mab (MIMA-9) with characteristics that allow for the simultaneous screening of RBCs of any ABO group for high-incidence antigen-negativity in the Kell and Gerbich blood group systems. MIMA-9, a murine IgG2a antibody, previously shown to facilitate the identification of K+k–, Kp(a+b–), K_0, McLeod, or Ge:–3 red blood cells (RBCs), was used in MTS gel cards containing anti-mouse IgG as the second antibody to test 1134 K– donors. Among the 1134 donors tested, we found one Kp(a+b–) and one Ge:–2,–3,4 donor. If random donor samples had been used instead of preselecting for K–, we would have expected to identify two K+k– donors. One reagent (MIMA-9) can be used to simultaneously screen for K+k–, Kp(a+b–), K_0, McLeod, and Ge:–3 RBCs and thereby conserve rare antisera. Inclusion of anti-mouse IgG in gel cards allowed for rapid screening. MIMA-9 is also a useful reagent to type RBCs with a positive direct antiglobulin test. This antibody is available to donor screening laboratories at no cost for this specific use. ***Immunohematology* 2002:18:43–45.**

Key Words: monoclonal antibody, anti-k, anti-Kp^b, rare donors

In 1975, Kohler and Milstein reported the first example of a murine hybridoma capable of secreting a murine monoclonal antibody (Mab).[1] Hybridomas are created by fusing a single stimulated B-cell (secreting an immunoglobulin with the specificity of interest) with a continuously growing myeloma cell. The resultant hybrid cell line secretes an antibody specific for a single epitope. Hybridoma technology has been applied widely in clinical and research laboratories.[2–4] We have produced new specificities using novel approaches. Since Mabs are potentially available in unlimited volume, they can be used to screen a large number of blood samples to identify antigen-negative donors. We have used a monoclonal antibody, MIMA-9 (Murine Immunochemistry Monoclonal Antibody), which we produced to screen donor red blood cells (RBCs) of any ABO group for several phenotypes.[5] MIMA-9 has an unusual specificity: the Mab reacts strongly with RBCs of common phenotype by the indirect antiglobulin test (IAT) using anti-mouse IgG but does not react with K_0 or Kp(a+b–) RBCs, and reacts only weakly with McLeod, K+k–, and some Ge-negative RBCs (because the majority of Ge:–2,–3,4 and Ge:–2,–3,–4 RBCs have depressed Kell antigen expression).[6]

Materials and Methods

The procedure for the production of this unusual murine IgG2a Mab (MIMA-9) has been described elsewhere.[3] Briefly, cDNAs encoding the common Kell antigens k, Kp^b, and Js^b were transfected into MEL-C88 cells by electroporation. The transfected clone with the highest expression of Kell antigens was selected as the immunogen. Standard hybridoma techniques were used to fuse mouse splenocytes with the mouse myeloma cell line X63.Ag8.653 to produce antibody-secreting hybridomas. The Mabs were characterized by serology and flow cytometry.

The pH preference of MIMA-9 was assessed by testing dilutions of the supernatant fluid in phosphate buffered saline (PBS) of different pH values by the IAT in tubes, using sheep anti-mouse IgG (The Binding Site, San Diego, CA). For subsequent testing, MIMA-9 was diluted in PBS/6% bovine serum albumin.

MIMA-9 was tested against 1134 donors of random ABO groups in experimental Micro-Typing System (MTS) gel cards (MTS, Pompano Beach, FL) especially filled by the manufacturer with rabbit anti-mouse IgG. Screening was performed by incubating 25 µL of the supernatant fluid containing Mab MIMA-9 and 50 µL of donor RBCs (0.8%) in the gel cards for 15 minutes at 37°C, and then centrifuging according to the manufacturer's directions. The donor RBCs tested in our study were preselected to be K–.

Results

Results of testing MIMA-9 at different pHs showed that this Mab is not dramatically sensitive to pH (Table 1). The results obtained using undiluted MIMA-9 supernatant fluid with selected RBCs in PBS at pH 7.3 are shown in Figure 1. At a dilution of 1 in 4 of the supernatant fluid containing MIMA-9 in PBS/6% bovine

Table 1. Results of testing MIMA-9 at different pHs

pH	IAT Titer*	Score
6.0	8	19
7.3	16	26
8.0	16	26

* Starting at a dilution of 1 in 2

serum albumin, comparable results were obtained; this dilution was selected for our screening. Among 1134 donors tested, we found one Kp(a+b–) and one Ge:–2,–3,4 donor. If random donor samples had been used instead of preselecting for K–, we would expect two K+k– donors to have been identified. To confirm the antigen-negative status, RBCs that were nonreactive in our initial screening using MTS gel cards were tested and confirmed with human polyclonal reagents by standard tube IAT.

Conclusion

The testing of 1134 donors with MIMA-9 detected one Kp(a+b–) donor and one Ge:–2,–3,4 donor.[5] In addition, had we used truly "random" donors, we would have expected to find two donors with K+k– RBCs. We previously showed that this one Mab, MIMA-9, can be used to detect K+k–, Kp(a+b–), K_0, McLeod, and Ge:–3 RBCs and thereby conserve rare antisera, which are not readily available for mass donor screening.[3] Inclusion of anti-mouse IgG in MTS gel cards allowed for rapid screening of large volumes of random donor RBCs. An additional advantage of this approach is that the Mab can be used for all ABO groups.

MIMA-9 is unusual in that, while it is nonreactive with Kp(a+b–) RBCs, it is only weakly reactive with K+k– RBCs. It was also surprising that Ge:–2,–3,4 and Ge:–2,–3,–4 RBCs were weakly reactive; however, it has been reported that most such RBCs have a weak expression of Kell antigens.[6] Thus, we conclude that the epitope recognized by MIMA-9 is highly conformation-dependent, is located on the Kell glycoprotein, and requires the presence of glycophorin C. We have taken advantage of this characteristic to perform mass screening with the Mab to identify several different high-incidence antigen-negative donors. Once we find a RBC sample that is nonreactive with MIMA-9, appropriate testing is performed to identify the actual phenotype of the donor. We will make MIMA-9 available to anyone who would like to include it in their screening program.

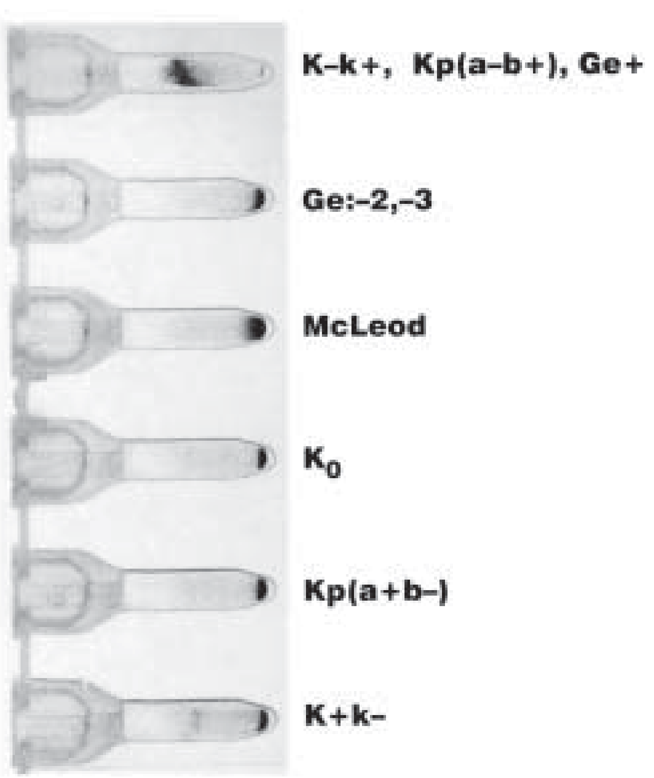

Fig. 1. MIMA-9 testing in an MTS gel card containing anti-murine IgG. RBCs with the following phenotypes: K+k–, Kp(a+b–), K_0, McLeod, Ge:–2,–3 are nonreactive. RBCs with the common phenotype (K–k+, Kp(a–b+), Ge+) are reactive.

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References

1. Kohler G, Milstein C. Continuous cultures of fused cells secreting antibody of predefined specificity. Nature 1975;256:495-7.
2. Chu T-HT, Halverson GR, Yazdanbakhsh K, et al. A DNA-based immunization protocol to produce monoclonal antibodies to blood group antigens. Br J Haematol 2001;113:32-6.
3. Chu T-HT, Yazdanbakhsh K, Øyen R, et al. Production and characterization of anti-Kell monoclonal antibodies using transfected cells as the immunogen. Br J Haematol 1999;106:817-23.
4. Halverson G, Chaudhuri A, Huang T, et al. Immunization of transgenic mice for production of MoAbs directed at polymorphic blood group antigens. Transfusion 2001;1393-6.

5. Tossas E, Øyen R, Reid ME, Malyska H. MIMA-9, a valuable antibody for screening for rare donors (abstract). Transfusion 2000;40(Suppl):126S.
6. Øyen R, Halverson GR, Reid ME. Review: conditions causing weak expression of Kell system antigens. Immunohematology 1997;13:75-9.

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Confirmation that the JAHK antigen is associated with the r^G haplotype

J. Kosanke, J.R. Storry, and M.E. Reid

Anti-JAHK, an antibody directed toward a low-incidence antigen in the Rh system, was detected during routine antibody identification in a male donor who had no history of transfusion. Examples of anti-JAHK have been found in sera containing multiple antibodies to low-incidence antigens. The first report of anti-JAHK was in 1995 and described the association of the JAHK antigen with the r^G haplotype. Our results confirm this association. ***Immunohematology* 2002;18:46–47.**

Key Words: low-incidence antigen, anti-JAHK, r^G haplotype, Rh blood group antigen

Green et al. first reported the JAHK antigen in 1995.[1] It was recognized as a new antigen when several sera containing multiple antibodies to other low-incidence antigens were reactive with red blood cells (RBCs) of two siblings. The RBCs from both siblings had a weak expression of the C antigen similar to that produced by the r^G haplotype [d(C)(e)G]. Family members who did not inherit the r^G haplotype were nonreactive with the new antibody. Another example of RBCs defined as r^Gr was also reactive, but RBCs that were r''^G were nonreactive. Two additional families were found with JAHK+ RBCs. In one family, the RBCs were studied because of weak expression of both C and e antigens in a person whose RBCs typed as D+C+E+c+e+. In the other family, the RBCs were studied because they reacted with a serum with multiple antibodies to low-incidence antigens. From observations in these three unrelated families, the JAHK antigen appeared to be produced by the r^G haplotype.

We report another example of anti-JAHK and confirmation of the association of this antigen with the r^G haplotype.

Case Report

A sample from a blood donor was referred to American Red Cross Blood Services, Central Ohio Region, for antibody identification. The sample was then sent to the New York Blood Center for further study. A panel of reagent RBCs was tested by a saline-indirect antiglobulin test (IAT). The donor's plasma, which contained anti-E and -C^w, reacted with one E–, C^w– RBC sample (donor T12) on the panel. These RBCs had the r^Gr phenotype.

Materials and Methods

Standard serologic techniques were used throughout. Reagent RBC panels were obtained from Immucor, Inc. (Norcross, GA); anti-IgG was from Gamma Biologicals, Inc. (Houston, TX). An aliquot of the incompatible panel RBCs (donor T12) was kindly provided by Immucor, Inc. To isolate the antibody and permit testing of additional RBCs regardless of ABO group, the donor's plasma was adsorbed onto an equal volume of r^Gr RBCs for 1 hour at 37°C. The antibody was eluted from these cells using a commercial kit (EluKit II, Gamma Biologicals, Inc.). This eluate was also tested with four r''^G RBC samples. The plasma was tested with RBCs known to express the following low incidence antigens: An^a, Co^b, Crawford, Dantu, Di^a, Go^a, Kp^a, Ls^a, Lu14, M^g, Mur, Hil, Js^a, Mt^a, Sc2, Rd, Tc^c, V, and Wr^a.

Results

The eluate prepared from the r^Gr RBCs after adsorption with the donor's plasma was reactive with all six r^G RBC samples and was nonreactive with the four r''^G RBCs. From these results, we concluded that the donor's plasma contains anti-JAHK. In addition to anti-E, -C^w, and -JAHK, antibodies to the low-incidence antigens Wr^a and M^g were identified in the donor's plasma.

Discussion

In the original study, anti-JAHK was present in several sera containing multiple antibodies to low-incidence antigens. The anti-JAHK reacted with those members of three unrelated families who inherited the r^G haplotype, suggesting that the JAHK antigen was associated with the r^G haplotype. Our case supports that association. In this case, the blood donor producing anti-JAHK denied any history of transfusion, which was confirmed by the hospital where his only known hospitalization occurred. Anti-E and -C^w were identified during that admission. These antibodies, along with anti-Wr^a, -M^g, and -JAHK identified in the current sample, appear to be naturally occurring. Thus, anti-JAHK has only been found in sera containing antibodies to multiple low-incidence antigens.

An eluate containing anti-JAHK reacted with four previously untested r^Gr RBC samples, providing further evidence that JAHK is an antigen carried by the r^G haplotype. Nonreactivity of the r''^G RBCs with the eluate confirmed the specificity.

Addendum

While this paper was in review, a current paper by Green et al.[2] reported that the ISBT has assigned Rh53 to the JAHK antigen.

References

1. Green CA, Lomas-Francis C, Wallace M, Bizoi M, Kasulke D, Goghlan G. Association between the r^G phenotype of the Rh system with a new low frequency antigen, JAHK (abstract). Transfus Med 1995;5(Suppl 1):19.
2. Green C, Coghlan G, Bizot M, et al. JAHK: a low frequency antigen associated with the r^G complex of the Rh blood group system. Transfus Med 2002;12:55-61.

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BOOK REVIEW

Practical Guide to Transfusion Medicine. Marian Petrides, MD, and Gary Stack, MD, PhD. Bethesda, MD: American Association of Blood Banks (AABB) Press, 2001. 366 pp. List Price: $105; Member/Student $75. ISBN 1-56395-128-2. To order call: (866) 222-2498.

Practical Guide to Transfusion Medicine is a useful, informative, and remarkably up-to-date textbook that is highly recommended to readers of *Immunohematology, Journal of Blood Group Serology and Education.* Drs. Marian Petrides and Gary Stack provide an overview of clinical transfusion practice, basic blood group immunohematology, and fundamentals of blood bank management in a readable and concise format. This paperback guide is now required reading for our medical center's Clinical Pathology residents, because it answers the most frequently asked questions in a hospital transfusion service with reliable and practical information.

The special interests of readers of *Immunohematology* are well-served by Dr. Stack's chapter on Carbohydrate-Based Blood Groups and Collections and a companion chapter on Peptide Blood Groups. Dr. Stack is a specialist in transfusion medicine who holds BS and PhD degrees in biochemistry, and who also completed a fellowship in molecular biology. He is expertly qualified and he presents blood group immunology with the precision, clarity, and detail that should satisfy the needs of laboratory-based immunohematologists. Dr. Petrides authored the chapters that address hemolytic disease of the newborn, neonatal alloimmune thrombocytopenia, platelet refractoriness, and calculations to determine the likelihood of finding compatible blood. The latter chapter contains a handy table of blood group antigen frequencies by race. Dr. Petrides adds useful guidance for estimating the frequencies of certain phenotypes which, because of their different distributions among populations according to race, do not segregate as truly independent variables.

A book reviewer should note deficiencies and, as expected, they can be found in any first edition, especially one that is crammed with technical information. The following sentence should be revised in the next edition: ". . . administration of RhIG or Rh IVIG is also recommended at 26–28 weeks of gestation for Rh-negative mothers when the father is Rh-negative or his Rh status is unknown" (pg. 280). Clearly, the authors intended "father is Rh-*positive*." There are (only) two photographic illustrations. The first is a photograph of a "plasma expresser." The priority for this photograph of laboratory equipment is unclear. The second is a reproduction of Immucor's widely-circulated illustration that compares the standard grading system for test tube agglutination reactions with scoring for Immucor's solid-phase red cell adherence assay (SPRCA). The SPRCA scores are barely discernible. A few pages later, the authors discuss SPRCA methodology using a line drawing as a model. However, the drawing of a "positive" reaction, which it describes as a uniform blush of indicator RBCs, is represented, erroneously, by a clear unshaded circle (pg. 39). The book has an errata sheet that identifies corrections needed for figures 3-7 and 5-8. Lastly, the Appendix, which is otherwise comprehensive and pertinent, provides the names and addresses for 13 journals intended to be "of interest to blood bank and transfusion medicine professionals." As I prepare this review, I feel obligated to note the absence of *Immunohematology,* a highly pertinent journal, from the list.

In balance, these few "first edition slips" are only minor distractions when weighted against the many contributions that *Practical Guide to Transfusion Medicine* will make educating trainees, providing a concise review for veteran blood bankers, and providing other healthcare professionals an informative, reliable, and affordable guide to transfusion practices.

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LITERATURE REVIEW

General (2000–2002)

Blood group antigens

1. Avent ND, Finning KM, Martin PG, Soothill PW. Prenatal determination of fetal blood group status. Vox Sang 2000;78:155-62.
2. Gubin AN, Njoroge JM, Wojda U, et al. Identification of the Dombrock blood group glycoprotein as a polymorphic member of the ADP-ribosyltransferase gene family. Blood 2000;96:2621-7.
3. Itoh Y, Kobayashi R. Evaluation of ABO and Lewis genotypes using primer extension preamplification. Forensic Sci Int 2000;113:139-41.
4. Jolly JG. Medicolegal significance of human blood groups. J Indian Med Assoc 2000;98:340-1.
5. Moulds JM, Zimmerman PA, Doumbo OK, et al. Molecular identification of Knops blood group polymorphisms found in long homologous region D of complement receptor 1. Blood 2001;97:2879-85.
6. Needs ME. RhD typing and the significance of Du. Br J Biomed Sci 2000;57:339.
7. Poole J. Red cell antigens on band 3 and glycophorin A. Blood Rev 2000;14:31-43.
8. Rupreht RR, Pretnar Hartman K, Galvani V, Rozman P, Curin Serbec V. Weak D and partial D in Slovenian population through serology and genotyping. Pflugers Arch 2000;440:195-6.
9. Storry JR, Lindsay G, Rolih S, et al. Four examples of anti-TSEN and three of TSEN-positive erythrocytes. Vox Sang 2000;79:175-9.
10. Storry JR, Reid ME, MacLennan S, Lubenko A, Nortman P. The low-incidence MNS antigens M(V), s(D), and Mit arise from single amino-acid substitutions on GPB. Transfusion 2001;41:269-75.
11. Zelinski T, Rusnak A, McManus K, Coghlan G. Distinctive Swann blood group genotype: molecular investigations. Vox Sang 2000;79:215-8.
12. Zizka Z, Calda P, Zlatohlavkova B, et al. Massive fetomaternal transplacental hemorrhage as a perinatal problem role of ABO fetomaternal compatibility—case studies. Med Sci Monit 2001;7:308-11.

Blood group antibodies

1. Ahaded A, Brossard Y, Debbia M, Lambin P. Quantitation determination of anti-K (KELL1) IgG and IgG subclasses in the serum of severely alloimmunized pregnant women by ELISA. Transfusion 2000;40:1239-45.
2. Beckers EA, van Guldener C, Overbeeke MA, van Rhenen DJ. Intravascular hemolysis by IgA red cell autoantibodies. Neth J Med 2001;58:204-7.
3. de La Rubia J, Arriaga F, Andreu R, et al. Development of non-ABO RBC alloantibodies in patients undergoing allogeneic HPC transplantation. Is ABO incompatibility a predisposing factor? Transfusion 2001;41:106-10.
4. Dhodapkar KM, Blei F. Treatment of hemolytic disease of the newborn caused by anti-Kell antibody with recombinant erythropoietin. Pediatr Hematol Oncol 2000;23:69-70.
5. Laine EP, Leger RM, Arndt PA, Calhoun L, Garratty G, Petz LD. In vitro studies of the impact of transfusion on the detection of alloantibodies after auto-adsorption. Transfusion 2000;40:1384-7.
6. Moise KJ. Non-anti-D antibodies in red cell alloimmunization. Eur J Obstet Gynecol Reprod Biol 2000;92:75-81.
7. Moran P, Robson SC, Reid M. Anti-E in pregnancy. BJOG 2000;107:1436-8.
8. O'Shea KP, Øyen R, Sausais L, et al. A MAR-like antibody in a $DC^{W}e/DC^{W}e$ person. Transfusion 2001;41:53-5.
9. Olujohungbe A, Hambleton I, Stephens L, Serjeant B, Serjeant G. Red cell antibodies in patients with homozygous sickle cell disease: a comparison of patients in Jamaica and the United Kingdom. Br J Haematol 2001;113:661-5.
10. Palfi M, Gunnarsson C. The frequency of anti-C plus anti-G in the absence of anti-D in alloimmunized pregnancies. Transfus Med 2001;11:207-10.
11. Reid ME, Sausais L, Øyen R, et al. First example of hemolytic disease of the newborn caused by anti-Or and confirmation of the molecular basis of Or. Vox Sang 2000;79:180-2.
12. Spong CY, Porter AE, Queenan JT. Management of isoimmunization in the presence of multiple maternal antibodies. Am J Obstet 2001;185:481-4.
13. Storry JR, Lindsay G, Rolih S, et al. Four examples of anti-TSEN and three of TSEN-positive erythrocytes. Vox Sang 2000;79:175-9.
14. Tearina Chu TH, Halverson GR, Yazdanbakhsh K, Øyen R, Reid ME. A DNA-based immunization protocol to produce monoclonal antibodies to blood group antigens. Br J Haematol 2001;113:32-6.

Blood group genetics

1. Chan FY, Cowley NM, Wolter L, et al. Prenatal *RHD* gene determination and dosage analysis by PCR: clinical evaluation. Prenat Diagn 2001;21:321-6.
2. Faas BH, Maaskant-Van Wijk PA, von dem Borne AE, Schoot CE, Christiaens GC. The applicability of different PCR-based methods for fetal RhD and K1 genotyping: a prospective study. Prenat Diagn 2000;20:453-8.
3. Itoh Y, Kobayashi R. Evaluation of ABO and Lewis genotypes using primer extension preamplification. Forensic Sci Int 2000;113:139-41.
4. Moulds JM, Zimmerman PA, Doumbo OK, et al. Molecular identification of Knops blood group polymorphisms found in long homologous region D of complement receptor 1. Blood 2001;97:2879-85.
5. Pramanik T, Pramanik S. Distribution of ABO and Rh blood groups in Nepalese medical students: a report. East Mediterr Health J 2000;6;156-8.
6. Rupreht RR, Pretnar Hartman K, Galvani V, Rozman P, Curin Serbec V. Weak D and partial D in Slovenian population through serology and genotyping. Pflugers Archiv 2000;440:195-6.
7. Yunis JJ, Yunis EJ, Yunis E. Genetic relationship of the Guambino, Paez, and Ingano Amerindians of southwest Columbia using major histocompatibility complex class II haplotypes and blood groups. Hum Immunol 2001;62:70-8.
8. Zelinski T, Rusnak A, McManus K, Coghlan G. Distinctive Swann blood group genotype: molecular investigations. Vox Sang 2000;79:215-8.

Monoclonal antibodies

1. Carbonnet F, Blanchard D, Hattab C, et al. A murine monoclonal antibody against Kx protein which reacts also with beta-spectrin. Transfus Med 2000;10:145-54.
2. Walker RY, Andrew S, Kumpel BM, Austin EB. Murine monoclonal antibodies reactive with a human monoclonal anti-RhD antibody (BRAD-5). Transfus Med 2000;10:225-31.
3. Williams M. Monoclonal reagents for rhesus-D typing of Irish patients and donors. Br J Biomed Sci 2000;57:142-9.

Red cell serology/methods

1. Ahaded A, Brossard Y, Debbia M, Lambin P. Quantitation determination of anti-K (KELL1) IgG and IgG subclasses in the serum of severely alloimmunized pregnant women by ELISA. Transfusion 2000;40:1239-45.
2. Baker JB, Korn CS, Robinson K, Chan L, Henderson SO. Type and crossmatch of the trauma patient. J Trauma 2001;50:878-81.
3. Chan FY, Cowley NM, Wolter L, et al. Prenatal *RHD* gene determination and dosage analysis by PCR: clinical evaluation. Prenat Diagn 2001;21:321-6.
4. Depkes D. Invasive versus non-invasive testing in red-cell alloimmunized pregnancies. Eur J Obstet Gynecol Reprod Biol 2000;92:83-9.
5. Faas BH, Maaskant-Van Wijk PA, von dem Borne AE, Schoot CE, Christiaens GC. The applicability of different PCR-based methods for fetal RhD and K1 genotyping: a prospective study. Prenat Diagn 2000;20:453-8.
6. Laine EP, Leger RM, Arndt PA, Calhoun L, Garratty G, Petz LD. In vitro studies of the impact of transfusion on the detection of alloantibodies after autoadsorption. Transfusion 2000;40:1384-7.
7. McCormick BA, Brown J, Davies R, Sanders DJ. Reducing unnecessary blood cross-matching. Anaesthesia 2001;56:377-8.
8. Ostendorf N, Niefhoff D, Cassens U, Sibrowski W. Automated serological compatibility testing using a solid-phase test and standard laboratory equipment. Vox Sang 2001;80:225-9.
9. Petrik J. Microarray technology: the future of blood testing? Vox Sang 2001;80:1-11.
10. Spindler JH, Kluter H, Kerowgan M. A novel microplate agglutination method for blood grouping and reverse typing without the need for centrifugation. Transfusion 2001;40:627-32.
11. Thorpe SJ, Turner CE, Heath AC, Sands D. A competitive enzyme-linked immunoassay using erythrocytes fixed to microtitre plates for anti-D quantitation in immunoglobulin products. Vox Sang 2000;79:100-7.
12. Wallis JP. Is it time to give up the crossmatch? J Clin Pathol 2000;53:73-5.
13. Wang Z, Shi J, Zhou Y, Ruan C. Detection of red blood cell-bound immunoglobulin G by flow cytometry and its application in the diagnosis of autoimmune hemolytic anemia. Int J Hematol 2001;73:188-93.
14. Yamada Y, Kobayashi M, Takatori T, et al. Possibility of paternity testing using RFLP analysis on a very small amount of material. J Forensic Odonto-stomatol 2001;19:1-4.

15. Zhong XY, Holzgreve W, Hahn S. Risk free simultaneous prenatal identification of fetal Rhesus D status and sex by multiplex real-time PCR using cell free fetal DNA in maternal plasma. Swiss Med Wkly 2001;131:70-4.

White cell/platelet serology

1. Bennett JS, Catella-Lawson F, Rut AR, et al. Effect of the P1(A2) alloantigen on the function of beta (3)-integrins in platelets. Blood 2001;97:3093-9.
2. Blumberg N, Heal JM, Hicks GL, Risher WH. Association of ABO-mismatched platelet transfusions with morbidity and mortality in cardiac surgery. Transfusion 2001;41:790-3.
3. Kankirawatana S, Kupatawintu P, Juji T, et al. Neonatal alloimmune thrombocytopenia due to anti-Nak(a). Transfusion 2001;41:375-7.
4. Klepfish A, Friedman J, Schechter Y, Schattner A. Autoimmune neutropenia, thrombocytopenia and Coombs positively in a patient with primary Sjogren's syndrome. Rheumatology 2001;40:48-9.
5. Manny N, Zelig O. Laboratory diagnosis of autoimmune cytopenias. Curr Opin Hematol 2000;7:414-9.
6. Navarrete CV. The HLA system in blood transfusion. Baillieres Best Pract Res Clin Haematol 2000;13: 511-32.
7. Panzer S. Report on the Tenth International Platelet Genotyping and Serology Workshop on behalf of the International Society of Blood Transfusion. Vox Sang 2001;80:72-8.
8. Tan J, Tang X, Xie T. Comparison of HLA class I typing by serology with DNA typing in a Chinese population. Transplant Proc 2000;32:1859-61.

Hemolytic anemias

1. Beckers EA, van Guldener C, Overbeeke MA, van Rhenen DJ. Intravascular hemolysis by IgA red cell autoantibodies. Neth J Med 2001;58:204-7.
2. Kerr R, Raiolinson PS, Cachia PG. Direct antiglobulin test negative, nonspherocytic autoimmune haemolytic anaemia. Clin Lab Haematol 2000;22:365-7.
3. Kuo PH, Yang PC, Kuo SS, Luh KI. Severe immune hemolytic anemia in disseminated tuburculosis with response to antituburculosis therapy. Chest 2001;119:1961-3.
4. Mullen CA, Thompson JN, Richard LA, Chan KW. Unrelated umbilical cord blood transplantation in infancy for mucopolysaccharidosis type 11B (Hunter syndrome) complicated by autoimmune hemolytic anemia. Bone Marrow Transplant 2000;25:1093-7.
5. Saif MW. HIV-associated autoimmune hemolytic anemia: an update. AIDS Patient Care STDs 2000; 15:217-9.
6. So CC, Wong KF, Yu PH, Kwan AM, Lee AW. Alloimmunization in Chinese with warm autoimmune haemolytic anaemia—incidence and characteristics. Transfus Med 2000;10:141-3.
7. Wang Z, Shi J, Zhou Y, Ruan C. Detection of red blood cell-bound immunoglobulin G by flow cytometry and its application in the diagnosis of autoimmune hemolytic anemia. Int J Hematol 2001;73:188-93.

Hemolytic disease of the newborn

1. Depkes D. Invasive versus non-invasive testing in red-cell alloimmunized pregnancies. Eur J Obstet Gynecol Reprod Biol 2000;92:83-9.
2. Dhodapkar KM, Blei F. Treatment of hemolytic disease of the newborn caused by anti-Kell antibody with recombinant erythropoietin. Pediatr Hematol Oncol 2000;23:69-70.
3. Goraya J, Basu S, Sodhi P, Mehta S. Unusually severe ABO hemolytic disease of the newborn. Indian J Pediatr 2001;68:285-6.
4. Haque KM, Rahman M. An unusual case of ABO-haemolytic disease of the newborn. Bangladesh Med Res Counc Bull 2000;26:61-4.
5. Maayan-Metzger A, Schwartz T, Sulkes J, Merlob P. Maternal anti-D prophylaxis during pregnancy does not cause neonatal haemolysis. Arch Dis Child 2001;84:pF60-2.
6. Naiman JL, de Alarcon PA. On Dr. Louis K. Diamond's 1932 article and subsequent contributions to erythoblastosis fetalis. J Pediatr Hematol Oncol 2001;23:373-6.
7. Narang A, Jain N. Haemolytic disease of the newborn. Indian J Pediatr 2001;68:167-72.
8. Reid ME, Sausais L, Øyen R, et al. First example of hemolytic disease of the newborn caused by anti-Or and confirmation of the molecular basis of Or. Vox Sang 2000;79:180-2.
9. Stockman JA, de Alarcon PA. Overview of the state of the art of Rh disease: history, current clinical management and recent progress. J Pediatr Hematol Oncol 2001;23:385-93.
10. Urbaniak SJ, Greiss MA. RhD haemolytic disease of the fetus and newborn. Blood Rev 2000;14:44-61.

11. Zhong XY, Holzgreve W, Hahn S. Risk free simultaneous prenatal identification of fetal Rhesus D status and sex by multiplex real-time PCR using cell free fetal DNA in maternal plasma. Swiss Med Wkly 2001;131:70-4.
12. Zizka Z, Calda P, Zlatohlavkova B, et al. Massive fetomaternal transplacental hemorrhage as a perinatal problem role of ABO fetomaternal compatibility—case studies. Med Sci Monit 2001; 7:308-11.

Transfusion/transplantation

1. Akkad A, Horworth E, Smith G, Scudamore I. To transfuse or not to transfuse: iatrogenic compromise of women's reproductive careers. Hosp Med 2001;62:310-1.
2. Bharucha ZS. Safe blood transfusion practices. Indian J Pediatr 2001;65:127-31.
3. Brand A. Immunological aspects of blood transfusion. Blood Rev 2000;14:130-44.
4. de La Rubia J, Arriaga F, Andreu R, et al. Development of non-ABO RBC alloantibodies in patients undergoing allogeneic HPC transplantation. Is ABO incompatibility a predisposing factor? Transfusion 2001;41:106-10.
5. Eckman JR. Technique for blood administration in sickle cell patients. Semin Hematol 2001;38:23-9.
6. Iturbe T, Cornudella R, Serrablo A, Gutierrez M. Adverse events associated with autologous and allogenic blood transfusion. J Bone Joint Surg 2000;82-A:1514-5.
7. Jadhav MV, Kurade N, Sahasrabudke N, Bepat VM. Blood transfusion associated fatalities. Ind J Pathol 2000;54:330-4.
8. Jeffries LC, Smith ME, Strobl FJ, Traber KB. Improved efficiency in providing blood to surgical patients using a novel approach to preadmission testing. Am J Med Qual 2000;15:251-6.
9. Klumper FJ, van Kamp IL, Vandenbussche FP, et al. Benefits and risks of fetal red-cell transfusion after 32 weeks gestation. Eur J Obstet Gynecol Reprod Biol 2000;92:91-6.
10. Lau FY, Wong R, Chui CH, Ng E, Cheng G. Improvement in transfusion safety using a specially designed transfusion wrist band. Transfus Med 2000;10:121-4.
11. Mielcarek M, Leisenring W, Torok-Storb B, Storb R. Graft-versus-host disease and donor-directed hemagglutin titers after ABO-mismatched related and unrelated marrow allografts: evidence for a graft-versus-plasma cell effect. Blood 2000;96:1150-6.
12. Mullen CA, Thompson JN, Richard LA, Chan KW. Unrelated umbilical cord blood transplantation in infancy for mucopolysaccharidosis type 11B (Hunter syndrome) complicated by autoimmune hemolytic anemia. Bone Marrow Transplant 2000;25:1093-7.
13. O'Brien BD. Blood transfusion requirements after liver biopsy. Can J Gastroenterol 2000;14:901-2.
14. Rios M, Hue-Roye K, Storry JR, Reiss RF. Cell typing the sensitized transfusion-dependent patients. Ann Clin Lab Sci 2000;30:379-86.
15. Rowley SD, Liang PS, Ulz L. Transplantation of ABO-incompatible bone marrow and peripheral blood stem cell components. Bone Marrow Transplant 2000;26:749-57.
16. Win N, Doughty H, Telfer P, Wild BJ, Pearson TC. Hyperhemolytic transfusion reaction in sickle cell disease. Transfusion 2001;41:323-8.
17. Young J. Transfusion reaction. Nursing 2000;30:33.

Disease associations

1. Garratty G. Blood group and disease: a historical perspective. Transfus Med Rev 2000;14:291-301.
2. Glinsky GV, Ivanova AB, Welsh J, McClelland M. The role of blood group antigens in malignant progression, apoptosis resistance and metatastic behavior. Transfus Med Rev 2002;14:326-50.
3. Klepfish A, Friedman J, Schechter Y, Schattner A. Autoimmune neutropenia, thrombocytopenia and Coombs positively in a patient with primary Sjogren's syndrome. Rheumatology 2001;40:48-9.
4. Kuo PH, Yang PC, Kuo SS, Luh KI. Severe immune hemolytic anemia in disseminated tuburculosis with response to antituburculosis therapy. Chest 2001;119:1961-3.
5. Olujohungbe A, Hambleton I, Stephens L, Serjeant B, Serjeant G. Red cell antibodies in patients with homozygous sickle cell disease: a comparison of patients in Jamaica and the United Kingdom. Br J Haematol 2001;113:661-5.
6. Saif MW. HIV-associated autoimmune hemolytic anemia: an update. AIDS Patient Care STDs 2000;15:217-9.
7. Timuragaoglu A, Duman A, Ongut G, Saka O, Karadogon I. The significance of autoantibodies in non-Hodgkin's lymphoma. Leuk Lymphoma 2000;40:1-2.

8. Ulvestad E, Berentsen S, Mollines TE. Acute phase hemolysis in chronic cold agglutinin disease. Scand J Immunol 2001;54:239-42.

Miscellaneous

1. Allain JP. Will genome detection replace serology in blood screening for microbial agents? Baillieres Best Pract Res Clin Haematol 2000;13:615-29.
2. Doubrovski VA, Dvoretski KN. Ultrasonic wave action upon the red blood cell agglutination in vitro. Ultrasound Med Biol 2000;26:655-9.
3. Eder AF, Manno CS. Does red-cell T activation matter? Br J Haematol 2001;114:25-30.
4. Quintana-Murci L, Krausz C, McElreavey K. The human Y chromosome: function, evolution and disease. Forensic Sci Int 2001;118:169-81.
5. Ringel PF, Weiler G, Bein G. Errors in ABO typing of blood stains using PCR. Int J Legal Med 2000;113:352-5.
6. Roberts AB, Mitchell JM, Lake Y, Pattison NS. Ultrasonic surveillance in red blood cell alloimmunization. Am J Obstet Gynecol 2001;184:1251-5.
7. Stott LM, Barker RN, Urbaniak SJ. Identification of alloreactive T-cell epitopes on the Rhesus D protein. Blood 2000;96:4011-9.
8. Zou CG, Agar NS, Jones GL. Haemolysis of human and sheep red blood cells in glycerol media: the effect of pH and the role of band 3. Comp Biochem Physiol 2000;127:347-53.

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