Immunohematology

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CONTENTS

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Evaluation and management of acute hemolytic transfusion reactions

L. Uhl and S.T. Johnson

A 53-year-old woman with persistent breast cancer after autologous bone marrow transplant 7 years previously for breast cancer was admitted to an outside institution because of gastrointestinal bleeding. Because of clinical symptoms related to anemia secondary to the gastrointestinal blood loss, a request for RBC transfusion was made. Routine serologic evaluation of the patient's blood sample demonstrated the presence of anti-c, and the patient was transfused with c–, crossmatch-compatible RBCs. As a result of ongoing transfusion requirements, the patient was transferred to our institution, at which time her plasma contained anti-c and anti-Jk^b. The patient was transfused with 4 units of c–, Jk(b–), RBCs that were crossmatch compatible by PEG (PeG, Gamma Biologicals, Inc., Houston, TX)-IAT (Gamma-clone Anti-IgG, Immucor/Gamma, Houston, TX) and sustained an appropriate rise in her hematocrit. The patient was discharged to home 48 hours after transfusion. Five days later she was readmitted with fever and recurrent anemia; a delayed hemolytic transfusion reaction (HTR) was suspected. Pertinent blood bank findings included the presence of anti-c (previously identified), anti-Jk^b (previously identified), and anti-s (new) (Table 1, Reaction 1). The patient's RBCs were positive by the DAT with anti-IgG (2+) (Gamma-clone Anti-IgG, Immucor/Gamma) and negative with anti-C3 (Gamma-clone Anti-C3b,C3d, Immucor/Gamma). Eluate analysis (Gamma ELU-KIT II, Gamma Biologicals, Inc.) demonstrated anti-s and anti-Jk^b. Because of ongoing hemolysis, the patient's Hct declined to 22%. A unit of RBCs was requested to manage symptomatic anemia. Owing to the complex antibody picture, specimens were referred to an immunohematology reference laboratory (IRL) to evaluate for additional antibody specificities. Testing revealed the presence of anti-c, anti-Jk^b, anti-s, probable anti-N, and probable HTLA (specificity undetermined); the presence of anti-K and anti-Fy^b could not be excluded. The DAT was negative using the most recent RBC sample. RBCs selected to lack c, K, s, Fy^b, Jk^b, and N were found to be weakly crossmatch incompatible at the antiglobulin (AHG) phase by the LISS tube method (ImmuAdd, Immucor/Gamma). The patient was transfused with 1 unit of RBCs without incident. However 2 hours after the transfusion, she developed a 2°F rise in temperature, chills, rigors, and dark urine.

This patient clearly manifested the hallmarks of an acute HTR, on the heels of a delayed HTR. HTRs are a consequence of RBC destruction and can be caused by an antibody-mediated process or a non–antibody-mediated process. The pathophysiology of the former is described here; the latter will be briefly reviewed later. Antibody-mediated acute HTRs occur when transfused RBCs bearing a foreign antigen are attacked by recipient antibodies directed against that antigen. ABO-incompatible transfusions are notorious for mediating acute HTRs, largely owing to the fact that the responsible antibodies are naturally occurring, or non–RBC stimulated (i.e., immune stimulation from a previous RBC transfusion is not required); are of the IgM class (which ably fixes complement); and are present in high titer. Non-ABO antibodies have been associated with acute HTRs, but in general these reactions are less severe (Table 2). The complex sequence of events after IgM antibody–associated complement activation is driven by the proteolytic cleavage of complement proteins. Through this

Table 1. Summary of transfusion reaction investigations

	Transfusion Reaction 1		Transfusion Reaction 2		Transfusion Reaction 3	
	Before	**After**	**Before**	**After**	**Before**	**After**
Visual inspection of plasma	Yellow	Amber	Yellow	Amber	Yellow	Orange
Serum antibodies	anti-c, anti-Jk^b	anti-c, anti-Jk^b, anti-s	anti-c, anti-Jk^b, anti-s, probable anti-N, HTLA	anti-c, anti-Jk^b, anti-s, HTLA	anti-c, anti-Jk^b, anti-s	anti-c, anti-Jk^b, anti-s*
DAT	Neg	IgG-2+, C3-neg	Neg	IgG- microscopically positive, C3-neg		Neg
Eluate		anti-s, anti-Jk^b		anti-s, anti-Jk^b		Neg
Hematocrit (%)	32	25	22	22	23	22
LDH (IU/L)	230	1,278	831	1,109	694	1,447
Haptoglobin (mg/dL)	101	<20	<20	<20	<20	<20
Indirect bilirubin (mg/dL)	0.1	3.9	0.2	0.9	0.2	3.1
BUN (mg/dL)	13	20	14	24	20	27
Creatinine (mg/dL)	0.7	0.8	0.7	0.9	1.7	1.6

*Anti-Do^a was identified by an immunohematology reference laboratory 1 week after the transfusion reaction event.
BUN = blood urea nitrogen

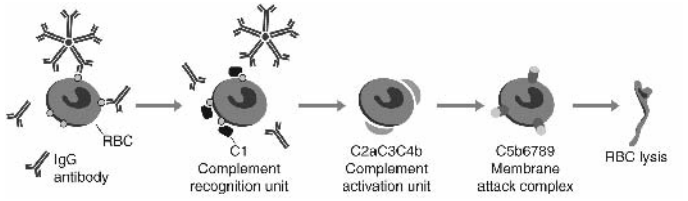

Fig. 1. Complement-mediated intravascular destruction of RBCs.

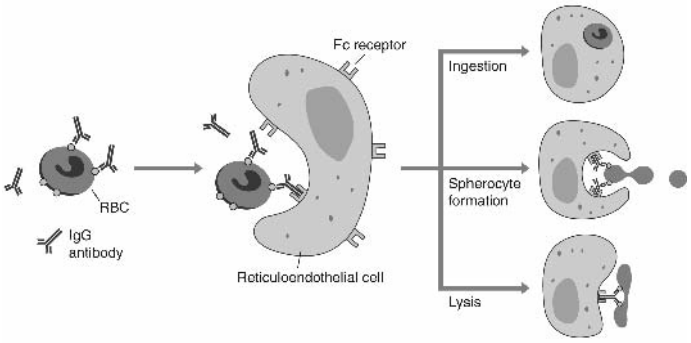

Fig. 2. IgG antibody-mediated extravascular destruction of RBCs.

activation process a host of biologic mediators are generated; in addition, the complement activation cascade promotes assembly of the pore-like membrane attack complex (C5b-9). Formation of the membrane attack complex on the surface of the RBC instigates intravascular lysis (Fig. 1).[1] The reticuloendothelial system also contributes to RBC destruction via erythrophagocytosis of complement (C3b)-coated RBCs.[2] In contrast, IgG-mediated antibody RBC destruction is thought to be largely extravascular. The fate of RBCs in this instance can take one of three paths, all presumably mediated by Fcγ receptors of splenic macrophages: (1) endocytotic removal of the sensitized RBC, (2) spherocyte formation as a result of membrane ingestion by the splenic macrophages, or (3) antibody-dependent cell-mediated cytolysis (ADCC; Fig. 2).[3]

The clinical manifestations observed in HTRs are secondary to cytokine generation after complement activation as well as cellular interaction between antibody- or complement-coated RBCs and phagocytic cells. Most notable are fever (attributable to increased production and release of interleukin 1 [IL-1], IL-6, IL-8, and tumor necrosis factor α) and pain at the intravenous site (likely related to complement proteins C3a and C3b).[4] Other less frequent symptoms include dyspnea, hypotension, nausea, and flushing.[5] Newly developed animal models of alloimmunization hold promise for further elucidation of the specific pathways involved in antibody-mediated RBC destruction and their physiologic consequences.[6,7] In a murine model using transgenic mice expressing human glycophorin A, Schirmer et al.[6] have examined the kinetics of IgM- and IgG-mediated removal of incompatible RBCs. Their results mirror previously reported chromium survival studies of antibody-mediated removal of RBCs performed in humans, thus supporting the model's potential to dissect the complexities of HTRs.[8] The investigators, using this model, hope to more clearly define the role of antibody class (IgM versus IgG) and IgG subclasses, complement proteins, and cellular Fcγ receptors in the pathophysiology of HTRs.[6]

Table 2. Complement-binding alloantibodies

Most	Some	Rare
ABO	-Le^b	-s
-Le^a	-S	-Fy^a
-Jk^a	-Xg^a	-Fy^b
-Jk^b	-LKE	
-P	-Lan	
-Pk		
-Vel		
-Ge		

In response to the patient's clinical symptoms of fever, chills and rigors, and hematuria after transfusion of the most recent RBC component, a transfusion reaction investigation was requested. A posttransfusion specimen and the requisition for transfusion reaction investigation were submitted. During the 2-hour interval between completion of the RBC transfusion and the patient's onset of symptoms, the blood component bag had been discarded into the hazardous waste container on the nursing unit, and therefore was unavailable to the blood bank laboratory for evaluation. On receipt of the post-transfusion specimen, the tube was centrifuged; examination of the plasma showed visual evidence of hemolysis (Table 1, Reaction 2). Repeat ABO typing (Gamma-clone, Immucor/Gamma) was performed and group A was confirmed. A DAT was performed, which demonstrated a microscopic positive reaction with anti-IgG (Gamma-clone Anti-IgG, Immucor/Gamma); no C3 was demonstrable using anti-C3 (Gamma-clone Anti-C3b,C3d, Immucor/Gamma) (with appropriate controls). An eluate analysis (Gamma ELU-KIT II, Gamma Biologicals, Inc.) demonstrated the presence of anti-s and anti-Jk^b.

In cases in which there is clinical concern for an HTR, a methodical approach to the laboratory evaluation is critical. The recent AABB publication Guidelines for the Laboratory Evaluation of Transfusion Reactions provides a useful resource for the evaluation of an HTR.[9] The authors describe a tiered approach to transfusion reaction investigation. The first tier includes a clerical check and three tests on a post-transfusion specimen: (1) visual check for evidence of plasma hemoglobinemia (Fig. 3), (2) repeat ABO typing [NB: Repeat ABO typing was not included in the Guidelines as the AABB Standards for Blood Banks and Transfusion Services, 22nd edition, added this testing to the first tier of investigation after publication of the guidelines in 2003.[10]], and (3) DAT. The clerical check is one of the most important steps for the exclusion of an ABO mistransfusion event as the cause of an HTR. It reviews for any errors in component labeling, including patient name and identifier (e.g., hospital record number), ABO group, and compatibility tags. In addition, the clerical check should include confirmation of the request for transfusion as well as prior transfusion history, transfusion restrictions, and the results of pretransfusion testing. After completion of the clerical check, a visual check for hemolysis in the posttransfusion specimen should be performed and, immediately afterward, an ABO determination. A discrepant ABO group in the posttransfusion specimen (when compared with the patient's historic type) raises the concern for an ABO-incompatible transfusion. In these instances, all units reserved for the patient in question should be quarantined, a request for another specimen for ABO typing should be made, and the blood bank medical director notified. If the transfusion reaction investigation reveals a positive DAT in the posttransfusion specimen, a DAT should be performed on a pretransfusion specimen to assess for an interval change. An antibody-mediated acute hemolytic transfusion event should be considered if an interval change is observed (i.e., pretransfusion DAT is negative and posttransfusion DAT is positive), and the

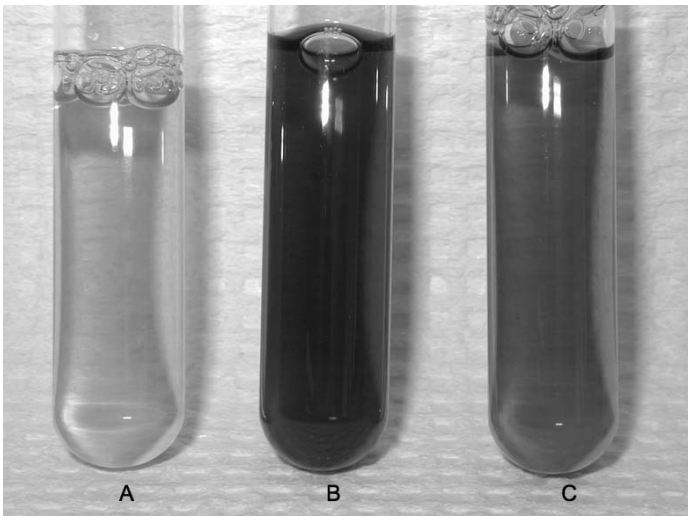

Fig. 3. Visual check for hemolysis. This image depicts the interval change in plasma color after a hemolytic transfusion reaction. The plasma from the pretransfusion specimen (Tube A) is yellow and clear. The plasma in the immediate posttransfusion specimen (Tube B) is red, consistent with the presence of free hemoglobin. Tube C depicts the interval color change secondary to metabolism of hemoglobin occurring during subsequent days after a hemolytic transfusion event.

laboratory should immediately take additional steps to clarify. As in the case in which an ABO transfusion error is suspected on the basis of a posttransfusion ABO type discrepancy, an interval change in the DAT should prompt the laboratory to quarantine reserved units and notify the blood bank medical director. Although a majority of individuals experiencing an acute HTR will have a positive DAT,[5] a negative DAT does not exclude the possibility of an immune-based HTR, particularly when there is evidence of plasma hemoglobinemia. This is most likely to occur in the setting of ABO-incompatible transfusion reactions, in which sensitized RBCs are rapidly removed from the circulation or hemolyzed.[11] In some settings in which the DAT is negative, further investigation via an RBC eluate analysis (considered to be third-tier testing) may provide clues to the cause of the HTR.

As noted previously, a positive (or discrepant) test result on a posttransfusion specimen is concerning and requires additional laboratory investigation. Suggested tests include repeat antibody screen, repeat crossmatch (carried through to the AHG phase of testing), repeat antigen typing of units in cases in which antigen-negative units were selected for transfusion, and evaluation of the RBC component for evidence of hemolysis (preferably the returned bag, otherwise unit segments are recommended).[9] The repeat antibody screen and crossmatch should be performed initially using the methods routinely used by the laboratory. If repeat testing is not illuminating, then use of more sensitive methods is recommended (e.g., PEG additive, enzymes, extended incubation). More sophisticated methods (third-tier testing) can be used in cases in which first- and second-tier testing is unrevealing as to the cause of the apparent immune HTR. Generally, these investigations are performed by IRLs and include adsorption-elution studies and enhanced antibody detection methods, including antibody neutralization methods, antibody titration, and recipient and donor antigen typing.[9] Genotyping, a tool recently introduced to the IRLs' armamentarium, can be particularly helpful in multiply transfused patients in whom it may be difficult to separate patient RBCs from transfused RBCs. In addition, genotyping may provide information on antigens in which there are limited antisera for typing available, for example, anti-Do^a and anti-Do^b. In cases in which the hunt for an unrecognized antibody is unrevealing, ancillary testing for other causes of hemolysis should be considered. These include flow cytometric analysis for CD59 to rule out paroxysmal nocturnal hemoglobinuria, evaluation for a Donath-Landsteiner antibody to rule out underlying paroxysmal cold hemoglobinuria,[12] and consideration of drug-induced immune hemolytic anemia.[13]

Other Considerations

When the laboratory transfusion investigation fails to uncover an immune cause for a clearly apparent HTR, it is important to exclude nonimmune causes of hemolysis.[12] One should exclude the possibility of osmotic RBC lysis secondary to use of an incompatible solution during transfusion (e.g., anything other than normal saline risks consequent hemolysis) or improper deglycerolization of a previously frozen unit. Exposure of an RBC component to temperature extremes (less than 0°C or greater than 40°C) may result in RBC lysis. Should concern for an HTR occur coincident with a surgical procedure or other therapeutic intervention in which blood is being passed through an extracorporeal circuit (e.g., hemodialysis or apheresis), mechanical injury to RBCs should be excluded as a cause of the apparent hemolytic event.

The transfusion medicine service was consulted regarding patient management after the episode of hemolysis, at which time they recommended gentle hydration and symptomatic treatment of fever with acetaminophen. Posttransfusion laboratory data (Table 1, Reaction 2) were remarkable for a Hct of 22% (unchanged from pretransfusion assessment), LDH of 1,109 U/dL (increased from pretransfusion assessment), haptoglobin of < 20 mg/dL (unchanged), and indirect bilirubin of 0.9 mg/dL (increased from 0.2 mg/dL). Analysis of a posttransfusion urine specimen was remarkable for dark (black) color, large concentration of blood by dipstick, and absent RBCs by microscopy. However, the patient's renal function variables were not significantly different from pretransfusion values (blood urea nitrogen: 14 mg/dL [pre], 24 mg/dL [post]; creatinine: 0.7 mg/dL [pre], 0.9 mg/dL [post]).

As illustrated by this case, assessment of hemolytic variables may be helpful in the diagnosis and determination of the magnitude of clinical impact of a presumed HTR. These include urinalysis, looking for evidence of hemoglobinuria (indicative of renal clearance of free hemoglobin derived from hemolyzed RBCs); increased LDH, an enzyme released into the

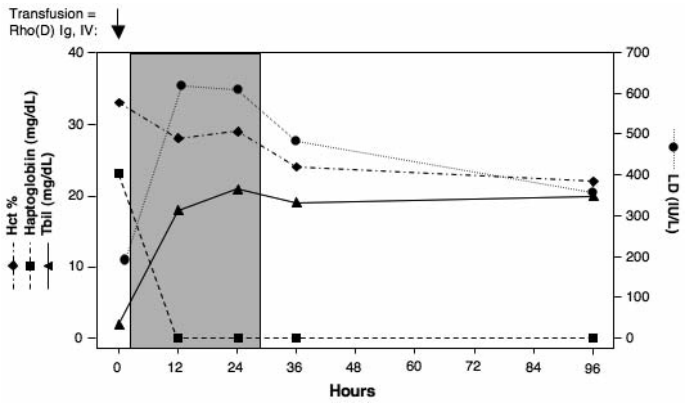

Fig. 4. Time course of change in laboratory variables of hemolysis after an acute hemolytic transfusion reaction. This graph depicts the temporal change in LDH (LD; ●), total bilirubin (Tbil; ▲), haptoglobin (■), and hematocrit (Hct; ◆) of a D+ patient with idiopathic thrombocytopenic purpura who experienced an acute hemolytic reaction after administration of Rh_0(D) immune globulin intravenous (WinRho SDF, Cangene Corporation, Winnipeg, Manitoba, Canada). Within hours of report of an acute hemolytic transfusion reaction, the patient's serum LDH and bilirubin rose, and haptoglobin dropped to undetectable levels. Not unexpectedly, there was a concomitant decline in the patient's hematocrit.

intravascular compartment after the destruction of RBCs (either intravascular or extravascular); indirect hyperbilirubinemia; and decreased haptoglobin as a consequence of increased clearance of heme-haptoglobin complexes.[14] Changes in these variables of hemolysis are generally evident within hours of the hemolytic event and normalize within days after completion of the RBC destruction process (Fig. 4).[15] For the patient suspected of having experienced an HTR, treatment strategies are empiric; because the potential magnitude of harm correlates with the volume of RBCs transfused, discontinuation of RBC transfusion is of paramount importance. Renal failure is one of the more serious risks of immune hemolysis. Consequently, treatment of hypotension with crystalloids is important to maintain renal function. Intravenous furosemide (20–80 mg) has been advocated to maintain renal tubular flow in hemodynamically stable patients.[16] The role of dopamine in low doses ("renal-dose" dopamine, 1–3 μg • kg–1 • min–1), which promotes renal vasodilation and increases urine output, to reduce the risk of anuric renal failure in the setting of HTRs is unclear, and, in general, it is not recommended.[17] Alkalinization of the urine with intravenous sodium bicarbonate (to a urine pH of >6.5) makes hemoglobin more soluble, and may prevent tubular obstruction by hemoglobin casts.[18] Massive release of intracellular RBC stores of potassium may produce critical hyperkalemia; thus, serum potassium concentrations should be monitored closely. Dialysis may be required in the setting of renal impairment to manage severe hyperkalemia. Rarely, disseminated intravascular coagulation (DIC) may occur in the setting of acute immune hemolysis as a result of procoagulant release during RBC destruction; this consumptive process may, in addition, be fueled by concomitant cytokine release. Similar to management of renal impairment, the treatment of DIC is supportive and based on the manifested abnormalities (e.g., transfusion of platelets for thrombocytopenia; cryoprecipitated AHF for hypofibrinogenemia, FFP for clotting factor deficiencies as assessed by prolongation of prothrombin time and activated partial thromboplastin time); heparin treatment is rarely necessary.

The patient remained stable after her second transfusion reaction. No additional transfusions were requested, and the patient was discharged to home with a Hct of 23.2%. With respect to the posttransfusion reaction serologic evaluation (Reaction 2), specimens referred to an IRL failed to demonstrate any additional clinically significant alloantibodies. The patient was advised to donate autologous blood components. With the support of erythropoietin therapy, she successfully donated four RBC units in the 6 months after the hemolytic transfusion event.

Future transfusion therapy should be based on the findings of the laboratory transfusion reaction investigation. In cases in which alloantibodies are identified, it is appropriate to select antigen-negative units that are crossmatched by an IAT. Autologous donation is a consideration for those patients for whom a transfusion reaction investigation demonstrates an antibody to a high-prevalence antigen, or, as in this case, transfusion therapy has been complicated by alloimmunization and recurrent transfusion reactions after transfusion with allogeneic blood. In such cases, patient referral to a regional donor center may be warranted so that units can be readily shipped in the event of an urgent need for RBC transfusion outside of the patient's locale.

The patient presented to the hospital on multiple occasions for management of anemia related to persistent gastrointestinal bleeding and bone marrow suppression secondary to ongoing palliative chemotherapy. Blood transfusion requirements were met using the patient's autologous units, all of which were transfused uneventfully. Because of progression of the

patient's disease, she was unable to continue autologous RBC donation, so when she was readmitted with symptomatic anemia after surgical stabilization of a pathologic fracture, a request was made for allogeneic blood transfusion. Once again, the serologic evaluation demonstrated anti-c, anti-Jk^b, and anti-s. Further evaluation by an IRL confirmed the presence of the alloantibodies, and recommendations were made to transfuse with antigen-compatible, least-incompatible RBCs. The patient received a single unit of RBCs lacking E, c, C^w, K, Jk^b, s, and Fy^a that was weakly incompatible by PEG (PeG, Gamma Biologicals, Inc.)-IAT but compatible by LISS (ImmuAdd, Immucor/Gamma)-IAT. The patient once again evidenced clinical symptoms of an HTR (fever, chills and rigors, and hematuria) after completion of the RBC transfusion (Table 1, Reaction 3). A transfusion reaction investigation was unrevealing, and specimens were referred to a second IRL for additional evaluation. This investigation led to the identification of anti-Do^a. The patient was subsequently successfully transfused with an RBC unit lacking Do^a, as well as E, c, K, Jk^b, and s.

The eventual identification of the anti-Do^a is not surprising.[19] This antibody is difficult to identify. It is often found in patients with multiple antibodies. It is weakly reactive, requiring enhancement methods such as PEG or testing with enzyme (ficin or papain)-treated RBCs. And finding reagent RBCs typed for Dombrock system antigens is difficult because of the rarity of potent, reagent-grade typing sera.

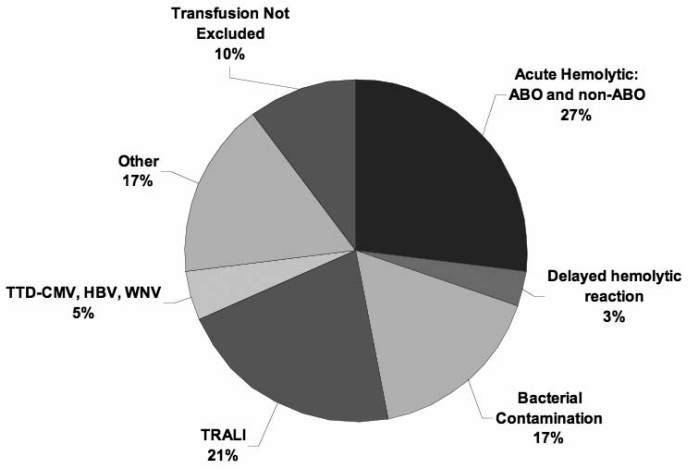

Fig. 5. Transfusion-associated fatalities reported to the U.S. Food and Drug Administration during the period of 2002 through 2004 (personal communication).

Closing Comments

Significant advances promoting overall transfusion safety, largely directed at the blood component itself, have been made during the last three decades. Most notable is the reduced risk of transfusion-transmitted viral infection.[20] Currently, the risk for transfusion-associated hepatitis C virus and HIV viral transmission is on the order of 1 in 2 million transfused units.[21] Nevertheless, blood transfusion is not risk-free. Non-infectious complications of transfusion, in particular hemolytic transfusion reactions, continue to be among the leading causes of transfusion-associated fatalities (Fig. 5).[22] Analysis of these events reproducibly shows that a majority of acute HTRs are a consequence of misidentification or incomplete identification of the transfusion recipient, at either the time of pretransfusion specimen acquisition or the time of blood component transfusion.[23] A much smaller proportion of HTRs, like the one described in this report, are caused by a failure to identify clinically significant alloantibodies. These observations have catalyzed the growing support for widespread adoption of hemovigilance programs within the United States. Such programs afford a mechanism by which robust data on transfusion complications and errors can be gathered and analyzed and, eventually, contribute to the development of innovative approaches to enhanced transfusion safety.[24,25] Although hemovigilance programs will improve transfusion safety, the challenge of detecting and identifying antibodies given current methods and the need for expertise in solving these types of problems remains.

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A novel study of association between *Neisseria gonorrhoeae* and the human carbohydrate blood groups

H.E. Perry, R.A. Franklin, S.J. Bray, M.K. Lo, L.A.C. Svensson, and S.M. Henry

Previous studies of association of ABO blood groups with gonorrhea have shown contradictory results. Despite the interdependencies of ABO, Lewis, and secretor systems, none of the previous studies examined the combined effect of these systems on their proposed association with gonorrhea. This study attempted to redress that and used genotyping in addition to RBC phenotyping to determine correct tissue phenotypes. Samples from 131 gonorrhea-positive individuals and from 175 gonorrhea-negative individuals were typed for ABO and Lewis using routine antisera. Secretor and Lewis genotyping was performed to ensure accurate determination of ABO and Lewis phenotypes. Chi-square and probability values were used to examine whether there is an association of ABO, Lewis, and secretor systems with gonorrhea infection. Neither single nor combined statistical analysis of data sets yielded a significant association of ABO, Lewis, and secretor phenotypes with *Neisseria gonorrhoeae.* Nevertheless, this study is an example of the approach that should be taken when examining microbial associations with ABO antigens, in turn influenced by coexpression and modification by the interdependent systems of Lewis and secretor, in mucosal tissues. ***Immunohematology* 2007; 23:100–104.**

This study examines whether there is an association between gonorrhea infection and the carbohydrate blood groups. Some microorganisms are known or believed to use specific carbohydrate receptors to invade the human mucosa.[1-4] Although the precise binding mechanism of microorganisms to mucosa is not well understood, it is probable that carbohydrate ligands such as those determined or modified by the blood group systems of ABO, secretor, and Lewis are involved.[5] Earlier studies have examined possible relationships between blood groups and gonorrhea, but are contradictory. Three separate studies reported gonorrhea to be more common in group B individuals.[6–8] However, three other similar studies found no relationship between ABO antigens and gonorrhea.[9–11]

Expression of ABO antigens in tissues is more complex than the simple expression of ABO antigens on RBCs.[12,13] Both Lewis and secretor genes have a major influence on the expression of blood group structures in mucosal tissues. Furthermore, it is established but poorly appreciated that typing RBCs is an inaccurate way of determining the Lewis phenotype of tissues. The reason for this is both the poor quality of Lewis reagents and the preference for conversion of precursor into ALe^b and BLe^b rather than Le^b in group A and B individuals.[5,12]

Consequently, determining the phenotype of RBCs alone cannot accurately predict the expression of the ABO and Lewis antigens in tissues. Determining the genetic status of the individual, as performed in this study, determines "true" tissue phenotypic expression of ABO, Lewis, and secretory antigens.

Table 1 summarizes the relative expression of antigens expected in mucosal tissues in some genotypes and phenotypes relevant to, and tested in, this study.

Materials and Methods

Attendees at Auckland Sexual Health clinics were invited to participate in the study (ethical approval; Auckland Ethics Committees 2001/113). The cohort consisted of 131 individuals who tested positive for gonorrhea (G+) at the District Health Board (DHB) laboratory, and 175 individuals who tested negative for gonorrhea (G–) at the DHB laboratory. Ethnicity was self-determined by participants into five groups (Maori, Pacific Island, Asian, New Zealand European, and other) and then coded by number so that ethnicity was known only by code (as required by the ethics committee).

Table 1. Constructed using available and unpublished data to compare and contrast relative expression of molecules on the tissues, and in the secretions, of individuals with different ABO, Le, and Se genetic profiles

	Relative antigen expression in secretions and mucosal surfaces*							
Genes present	**Le^c**	**Le^d**	**Le^a**	**Le^b**	**A-1/B-1**	**ALe^b**	**BLe^b**	**RBC phenotype**
A, Le, Se	(+)	(+)	+	++	++	++++		A Le(a–b+)
B, Le, Se	(+)	(+)	+	++	++		++++	B Le(a–b+)
O, Le, Se	(+)	+	+	++++++				O Le(a–b+)
ABO, Le, sese	+		++++++					ABO Le(a+b–)
A or *B, lele, Se*	(+)	++			+++++			A or B Le(a–b–)
O, lele, Se	(+)	++++++						O Le(a–b–)
ABO, lele, sese	++++++++							ABO Le(a–b–)

* Le^c = type 1 precursor; Le^d = H type 1; A-1/B-1 = either A type 1 or B type 1
(+) Very low expression
+, ++ Low expression
++++, +++++, ++++++ Moderate expression
++++++++ High expression

One 10-mL tube of blood, anticoagulated with CPD, was collected from each participant. Blood samples were centrifuged at 1000 × *g* for 20 minutes to separate plasma, WBCs, and RBCs. ABO and Lewis typing and DNA extraction were performed on the centrifuged sample. ABO RBC and plasma blood grouping with commercial antisera (monoclonal anti-A and anti-B, Commonwealth Serum Laboratories, Australia) and reagent RBCs was performed by standard saline tube method. Serologic Lewis typing was performed with commercial antisera (monoclonal anti-Le^a and anti-Le^b, Ortho-Clinical Diagnostics, Raritan, NJ) and unwashed RBCs by a standard saline tube method. Lewis genotyping was performed on all samples with Le(a–b–) phenotypes. Secretor status was determined by genotyping.

Extracted DNA was subjected to PCR amplification for genotyping. Secretor genotyping was performed using an established method capable of detecting a range of mutations including Se^w.[14] Participants were classified as either "secretor," "nonsecretor," or "partial secretor." Lewis genotyping by an established PCR sequence-specific primer method[15] was undertaken on samples from 32 individuals whose RBCs typed as Le(a–b–). The Lewis-negative status was assigned to samples proven to lack *Le* by genotyping.

Phenotypes reported and used for analyses were based on a combination of serologic phenotypes and supportive genotypes. Where there was a discrepancy between the RBC phenotype and the genotype, the genotype took preference.

Chi-square and probability values were used to examine whether there is an association of ABO, Lewis, and secretor blood group–related phenotypes with gonorrhea infection, both in isolation and in a set of five hypotheses based on expression of different carbohydrate molecules, as a result of the different gene combinations. The hypotheses are that Le^a, Le^b, Le^c, Le^d, and ABO antigens are predisposing factors to infection with *Neisseria gonorrhoeae.*

Results

The characteristics of the study cohort are shown in Table 2. As previously reported,[16] there is a significant statistical difference between ethnic distribution in the G+ and G– groups ($p < 0.001$).

ABO, Lewis, and secretor genotypes were determined for all individuals (Table 3). A simple comparison of the association of G+ with the RBC–defined ABO group revealed no statistically significant difference between ABO blood groups and gonorrhea infection alone ($p = 0.95$; Table 3). As individuals that type as group B have been reported to

Table 2. The study cohort

	G+ group	**G– group**
Gonorrhea status	Positive	Negative
Number of individuals	131	175
Age (years)	14-60	15-64
Male	93	125
Female	36	49
Gender not stated	2	1
Ethnic group 1	21	7
Ethnic group 2	59	123
Ethnic group 3	5	19
Ethnic group 4	35	17
Ethnic group 5	6	8
Ethnicity not stated*	5	1

*Participants not used for statistical analysis requiring ethnicity.

Table 3. ABO, Lewis, and secretor distribution in G+ and G– groups (all ethnicities)

	ABO				Secretor			Lewis			
	A	**B**	**AB**	**O**	**Se**	**NS**	**Se^w**	**a–b+**	**a+b+**	**a+b–**	**a–b–**
G+	52 (40%)	15 (11%)	7 (5%)	57(44%)	75 (57%)	21 (16%)	35 (27%)	74 (56%)	33 (25%)	16 (12%)	8 (6%)
G–	64 (36%)	21 (12%)	10 (6%)	80 (46%)	113 (65%)	35 (20%)	27 (15%)	109 (62%)	26 (15%)	27 (15%)	13 (7%)
χ^2	0.31				6.0			5.31			
p	0.95				0.05			0.15			

Se = secretor (genotypes *SeSe, SeSe^w, Sese*); NS = nonsecretor (genotype *sese*); Se^w = partial secretor (genotypes *Se^{w}Se^w, Se^{w}se*)

be linked with gonorrhea infection in previous studies,[6–8] the significance of an individual typed as group B was reexamined. Group B individuals, alone or when combined with group AB individuals, were tested against individuals of all other blood types, and the results were not significant (p = 0.68 and p = 0.58, respectively).

There was no statistically significant difference between RBC Lewis phenotypes and gonorrhea status (p = 0.15; Table 3).

The relationship between secretor status in the G+ and G– cohorts initially showed statistically significant differences related to the partial secretor phenotype (p = 0.05; Table 3). However, as the *Se^w* gene is not expressed in ethnic group 2, the data were reanalyzed in Se^w-expressing ethnic groups and there was no statistical significance (p = 0.33).

None of the results of hypotheses 1, 2, 3, and 5 showed any association between gonorrhea and combinations of blood group expression on the mucosal tissues (Tables 4–7). There were insufficient data to test hypothesis 4.

Discussion

Although previous reports in the literature have been ambiguous, the possibility that the microorganism *N. gonorrhoeae* could use carbohydrate blood group antigens as receptors, and thus show a blood group association, is reasonable. Blood group antigen associations have been postulated for many microorganisms, and there is some biochemical evidence to support this.[4,5,17] Regrettably, the determination of blood groups in tissues is much more complex than the simple determination of the RBC phenotype, and many blood group associations have been clouded by incorrect phenotyping.[5] The expression of ABO blood group antigens is controlled by inheritance of ABO genes and the polymorphic secretor gene and modified by the expression of the polymorphic Lewis gene.[12,13] These complex interactions are well known today, yet almost all prior surveys have failed to account for these interactions, which significantly determine the type and quantity of molecules expressed in the tissue of interest (Table 1). The issues of studying a disease and blood group associations are further compounded by the inability to obtain accurate Lewis and secretor genotypes from RBC typing, and racial variations.[18] Error rates in typing for these systems are high,[12] and higher in diseased populations or in some ethnic groups; thus genotyping is essential to determine accurate secretor and Lewis types. The association of a disease with the ABO blood group system can only be determined if the study includes measures to accurately determine ABO, Lewis, and secretor tissue phenotypes.

We determined blood groups and analyzed samples from 131 individuals who were identified as being infected with gonorrhea (G+) against 175 gonorrhea-uninfected (G–) individuals as control subjects. The G– group was selected on the basis of exclusion for gonorrhea positivity, and was therefore not a true randomly selected control group. However, this control group was used rather than published data from blood donor studies because it was believed the control group from the clinic would be a better control of socioeconomic and ethnic factors. It was essential to control for ethnicity in the study, as different ethnic groups have different blood group gene frequencies, and different rates of infection with gonorrhea. Although we were blinded with regard to ethnicity, we were able to compare and contrast the test data with ethnic considerations.

There was as expected[12] a high rate of discordance (17%) between Lewis RBC phenotypes (often used to predict secretor status) and Lewis and secretor genotypes. Genotypes were therefore used for data analysis rather than the serologic phenotypes.

Extensive analysis found no association of G+ infection with blood groups ABO, Lewis, or secretor in ethnic populations based in New Zealand. This study was extensive in that it fully considered the factors related to blood group expression and association with

Table 4. Hypothesis 1. Comparison of high expression of Le^a in all ABO groups with no expression of Le^a in all ABO groups

	ABO Le(a+b–)	vs	ABO and Le(a–b–)
ABO	All		All
Lewis	Positive		Negative
Secretor	Negative		All
G+	16		8
G–	26		12
χ^2	0.021		
p	0.886		

Table 5. Hypothesis 2. Comparison of high expression of Le^b in O Le(a–b+) with no expression of Le^b in all ABO groups

	O Le(a–b+)	vs	ABO + Le(a+b–) and Le(a–b–)
ABO	O		All
Lewis	Positive		Combinations producing no Le^b
Secretor	Positive		
G+	34		24
G–	54		42
χ^2	0.083		
P	0.773		

Table 6. Hypothesis 3. Comparison of high expression of Le^c in Le(a–b–) sese (all ABO groups) with low expression of Le^c in Le(a–b+) (all ABO groups)

	ABO Le(a–b–) sese	vs	ABO Le(a–b+)
ABO	All		All
Lewis	Negative		Positive
Secretor	Negative		Positive
G+	5		73
G–	7		109
χ^2	0.011		
p	0.915		

Table 7. Hypothesis 5. Comparison of expression of A, B, and H in secretors

	A Se	B Se	AB Se	O Se
ABO	A	B	AB	O
Lewis	All	All	All	All
Secretor	Positive	Positive	Positive	Positive
G+	29	7	4	34
G–	33	14	9	57
χ^2	2.294			
P	0.514			

infection. Future studies could be further strengthened if consideration was also given to serotyping or genotyping the microorganisms involved and larger numbers of samples were available. Although blood group associations with disease remain tantalizing, it appears that *N. gonorrhoeae* is not an organism that has an association with ABO, Lewis, or secretor antigens.

Acknowledgments

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An update on drug-induced immune hemolytic anemia

G. Garratty and P.A. Arndt

Four previous reviews for this journal (1985,[1] 1989,[2] 1994,[3] 2004[4]) and 3 others published more recently[5–7] give a good sense of the changes that have occurred in the last 20 years in the study of drug-induced immune hemolytic anemia (DIIHA). These changes include new drugs that cause DIIHA, incidence of the involvement of certain drugs, revisions to proposed mechanisms involved in DIIHA, and new information on individual drugs. In our review in 2005,[6] we emphasized the changing spectrum of drugs associated with DIIHA. In the 10-year period 1969 to 1979, methyldopa was responsible for 67 percent, and high-dose intravenous penicillin for 23 percent, of the DIIHAs we encountered. In the 1995 to 2004 period, 82 percent of DIIHAs were associated with second- and third-generation cephalosporins (72% of these were associated with cefotetan, and 10% with ceftriaxone).

Drug-induced antibodies are of two types: (1) drug-independent antibodies (no drug is needed to demonstrate the presence of antibody, i.e., react similarly to true autoantibodies); and (2) drug-dependent antibodies, which require drug to be present to demonstrate the antibody. The drug can be covalently bound to the RBC membrane, or exist free in the plasma. Thus, drug-dependent antibodies can be demonstrated by testing the patient's plasma or serum, or an eluate from DAT-positive RBCs with RBCs coated with drug in vitro, or testing the serum in the presence of a drug solution and RBCs or both.

In Tables 1 and 2, we list 125 drugs that we believe have reasonable evidence to support a drug-associated immune etiology for DIIHA or positive DATs.[8–151] We cannot include every reference, so we selected the first report that contained reasonable data and further references that added important extra data. Table 1 contains a list of 108 drugs in which drug-dependent antibodies (i.e., antibodies that only react with RBCs when drug is present, either bound to the RBCs, or when added to the patient's serum, and target RBCs[8–124]) were detected. Twenty-six percent of the drugs were associated with antibodies that only reacted with drug-coated RBCs; 39 percent were associated with antibodies that only reacted in the presence of drug, and 35 percent were associated with antibodies that reacted with drug-treated RBCs or in the presence of drug or both. One surprising result was that 44 percent of these reactions were accompanied by reactions against untreated RBCs without the addition of drugs in vitro. We believe that these reactions are not caused by drug-independent autoantibodies such as those listed in Table 2, but rather are a subpopulation of drug-dependent antibodies reacting with the drug plus RBC membrane proteins and not needing drug for their demonstration, or are caused by circulating drug, or drug–anti-drug complexes (see later).

Figure 1 shows the concept of the unifying hypothesis,[5,67,152,153] based on Landsteiner's work on antibody populations induced by haptens (small molecules such as drugs).[154] One population of antibodies reacts with the hapten (drug) alone; another population reacts with part drug plus carrier (protein on RBC membrane). This epitope may be mostly the carrier as illustrated on the left side of the cartoon. Antibodies that react with drug alone can be detected using RBCs coated with the drug. Some investigators call this the "hapten mechanism." This makes no sense to us as all drugs are haptens (i.e., small molecules that require a carrier such as a protein to be immunogenic). The term was first used to describe the penicillin antibody reactions, but at that time few drug antibodies had been described and little was known of drug immunology. (For further discussion of this see pages 263–7 of Petz and Garratty.[5]) Antibodies reacting with an epitope such as the one illustrated on the bottom right side of the cartoon will react when the patient's serum is mixed with drug and RBCs (this mechanism is sometimes called the "immune-complex" mechanism). When the epitope is mainly composed of the RBC membrane, then the antibody may react with RBCs without any drug being present and appears to be a

Table 1. Drugs associated with cases of IHA or positive DAT or both in which drug-dependent antibodies were detected*

Drug (Alternative name)	Reference	Therapeutic category	Number of references [single (year) vs. multiple (<5, <10, ≥10)]	HA	Positive DAT	Method of detecting serum antibody			Reactive without drug added in vitro
						Drug-coated RBCs	Serum + drug + RBCs	Not reported	
Aceclofenac	8	NSAID	Single (1997)	✔	✔	–	✔	–	–
Acetaminophen (Paracetamol)	9, 10	NSAID	Multiple (<10)	✔	✔	–	✔	–	–
Acyclovir	11	Antiviral	Single (2003)	✔	✔	✔	–	–	–
Aminopyrine (Piramidone)	12	NSAID	Single (1961)	✔	–	✔	–	–	–
Amoxicillin	13	Antimicrobial	Single (1985)	✔	✔	✔	–	–	–
Amphotericin B	14	Antimicrobial	Multiple (<5)	✔	✔	–	✔†	–	–
Ampicillin	15	Antimicrobial	Multiple (<10)	✔	✔	✔	✔	–	–
Antazoline	16	Antihistamine	Multiple (<5)	✔	✔	–	✔	–	–
Aspirin	17	Analgesic, antipyretic, anti-inflammatory	Single (1984)	✔	–	–	✔	–	–
Azapropazone (Apazone)	18	Antiinflammatory, analgesic	Multiple (<5)	✔	✔	✔	–	–	✔
Buthiazide (Butizide)	19	Diuretic, antihypertensive	Single (1984)	✔	✔	–	✔†	–	–
Carbimazole	20	Antithyroid	Multiple (<5)	✔	✔	✔	✔	–	✔
Carboplatin‡	21	Antineoplastic	Multiple (<5)	✔	✔	✔	✔	–	✔
Carbromal	22	Sedative, hypnotic	Single (1970)	–	✔	✔	–	–	–
Catechin ((+)-Cyanidanol-3) (Cianidanol)	23	Antidiarrheal	Multiple (≥10)	✔	✔	✔	✔†	–	✔
Cefamandole	24	Antimicrobial	Single (1985)	✔	✔	✔	–	–	–
Cefazolin	25	Antimicrobial	Multiple (<10)	✔	✔	✔	–	–	–
Cefixime	26	Antimicrobial	Single (2000)	✔	–	✔	✔	–	–
Cefotaxime‡	27	Antimicrobial	Multiple (<5)	✔	✔	✔	✔	–	✔**
Cefotetan‡	28–32	Antimicrobial	Multiple (≥10)	✔	✔	✔¶	✔	–	✔
Cefoxitin‡	33	Antimicrobial	Multiple (<10)	✔	✔	✔	✔	–	✔
Cefpirome	34	Antibacterial	Single (2005)	–	✔	–	✔	–	–
Ceftazidime	35	Antimicrobial	Multiple (<10)	✔	✔	✔	✔	–	✔
Ceftizoxime	36	Antimicrobial	Multiple (<5)	✔	✔	✔	✔	–	✔**
Ceftriaxone‡	37, 38	Antimicrobial	Multiple (>10)	✔	✔	–	✔†	–	✔**
Cefuroxime	39	Antibacterial	Multiple (<5)	✔	✔	✔	–	–	–
Cephalexin	40	Antimicrobial	Multiple (5)	✔	✔	✔¶	–	–	–
Cephalothin‡	41–43	Antimicrobial	Multiple (≥10)	✔	✔	✔¶	✔	–	–
Chloramphenicol	44	Antibacterial	Multiple (<5)	✔	✔	✔	–	–	✔
Chlorinated hydrocarbons	45	Insecticides	Multiple (<10)	✔	✔	✔	✔	–	✔
Chlorpromazine	46	Antiemetic, antipsychotic	Multiple (<10)	✔	✔	✔	–	–	✔
Chlorpropamide‡	47, 48	Antidiabetic	Multiple (<10)	✔	✔	–	✔	–	✔**
Ciprofloxacin	49	Antibacterial	Multiple (<10)	✔	✔	–	✔	–	✔
Cisplatin (Cisdiamino-dichloroplatinum)	50, 51	Antineoplastic	Multiple (<10)	✔	✔	✔¶	✔	–	–
Cloxacillin	52	Antibacterial	Single (1980)	–	✔	–	–	✔	✔
Cyclofenil	53	Gonad-stimulating principle	Multiple (<5)	✔	✔	–	✔	–	✔
Cyclosporin (Cyclosporine)	54	Immunosuppressant	Multiple (<5)	✔	✔	✔	–	–	✔

Drug (Alternative name)	Reference	Therapeutic category	Number of references [single (year) vs. multiple (<5, <10, ≥10)]	HA	Positive DAT	Method of detecting serum antibody			Reactive without drug added in vitro
						Drug-coated RBCs	Serum + drug + RBCs	Not reported	
Dexchlorpheniramine maleate (Chlorpheniramine)	55	Antihistaminic	Single (1981)	✔	✔	–	✔	–	–
Diclofenac‡	56–59	NSAID	Multiple (≥10)	✔	✔	✔	✔†	–	✔**
Diethylstilbestrol (Stilboestrol)	60	Estrogen	Multiple (<5)	✔	✔	–	✔	–	–
Dipyrone	61	NSAID	Multiple (<5)	✔	✔	✔	✔	–	–
Erythromycin‡	62	Antimicrobial	Multiple (<5	✔	✔	✔	–	–	–
Etodolac	63	NSAID	Single (2000)	✔	✔	–	✔†	–	–
Ethambutol	11	Antibacterial	Single (2003)	✔	✔	✔	✔	–	–
Fenoprofen	64	NSAID	Single (1988)	✔	✔	–	✔	–	✔**
Fluconazole	11	Antifungal	Single (2003)	✔	✔	✔	✔	–	–
Fluorescein	65	Injectable dye	Single (1993)	✔	✔	✔	✔	–	✔**
Fluorouracil	66	Antineoplastic	Multiple (<5)	✔	✔	–	✔	–	–
Furosemide	35	Diuretic	Multiple (<5)	–	✔	–	✔	–	–
Glafenine (Glaphenine)	67, 68	Analgesic	Multiple (<5)	✔	✔	–	–	✔†	✔
Hydralazine	69	Antihypertensive	Single (1977)	✔	✔	✔	–	–	–
Hydrochlorothiazide‡	70	Diuretic	Multiple (<10)	✔	✔	✔	✔	–	✔**
9-Hydroxy-methyl-ellipticinium (Elliptinium acetate)	71	Antineoplastic	Multiple (<5)	✔	✔	–	✔	–	–
Ibuprofen	72	NSAID	Multiple (<5)	✔	✔	–	✔	–	✔
Imatinib mesylate	73	Antineoplastic	Multiple (<5)	✔	✔	✔	–	–	–
Insulin	74	Antidiabetic	Multiple (<5)	✔	✔	✔	–	–	–
Isoniazid	75	Antimicrobial	Multiple (<10)	✔	✔	✔	✔	–	–
Latamoxef (Moxalactam)	67	Antimicrobial	Single (1985)	✔	✔	–	–	✔	✔
Levofloxacin (Ofloxacin)	76	Antibacterial	Multiple (<5)	✔	✔	✔	✔	–	✔
Mefloquine‡	77	Antimicrobial	Multiple (<5)	✔	✔	✔	✔	–	✔**
Melphalan	78	Antineoplastic	Single (1967)	✔	–	–	✔	–	–
6-Mercaptopurine	79	Anti-neoplastic	Single (2000)	✔	✔	✔	–	–	–
Methadone	80	Analgesic	Multiple (<5)	–	✔	✔	–	–	–
Methotrexate	81	Antineoplastic, antirheumatic	Multiple (<5)	✔	✔	✔	✔	–	✔
Metrizoate-based radiographic contrast media	82		Multiple (<5)	✔	✔	✔	✔	–	✔
Minocycline	83	Antibacterial	Single (1994)	✔	✔	–	✔	–	–
Nabumetone analgesic	84	Antiinflammatory,	Single (2003)	✔	✔	–	✔†	–	✔
Nafcillin‡	85	Antimicrobial	Multiple (<10)	✔	✔	✔	–	–	–
Naproxen	86	Antiinflammatory, analgesic, antipyretic	Multiple (<5)	✔	✔	–	✔	–	–
Nitrofurantoin	87	Antibacterial	Single (1981)	✔	–	–	✔	–	–
Nomifensine§	88	Antidepressant	Multiple (≥10)	✔	✔	–	✔†	–	✔**
Norfloxacin	89	Antimicrobial	Single (1999)	–	✔	✔	–	–	–
Oxaliplatin‡	90, 91	Antineoplastic	Multiple (≥10)	✔	✔	✔¶	✔	–	✔**
p-Aminosalicylic acid (PAS) (para-aminosalicylsaure)	92	Antimicrobial	Multiple (<10)	✔	✔	–	✔	–	–

Drug (Alternative name)	Reference	Therapeutic category	Number of references [single (year) vs. multiple (<5, <10, ≥10)]	HA	Positive DAT	Method of detecting serum antibody			Reactive without drug added in vitro
						Drug-coated RBCs	Serum + drug + RBCs	Not reported	
Penicillin G‡	93, 94	Antimicrobial	Multiple (≥10)	✔	✔	✔	✔	–	–
Phenacetin‡ (Acetophenetidin)	95	NSAID	Multiple (≥10)	✔	✔	–	✔	–	✔
Phenytoin (Fenitoine)	11	Anticonvulsant, antiarrhythmic	Single (2003)	✔	✔	✔	–	–	–
Piperacillin‡	96	Antimicrobial	Multiple (<10)	✔	✔	✔	✔	–	✔**
Probenecid‡	97	Uricosuric	Multiple (<5)	✔	✔	–	✔	–	✔**
Propyphenazone	98	NSAID	Single (1998)	✔	✔	–	✔	–	–
Pyrazinamide	11	Antibacterial	Single (2003)	✔	✔	✔	✔	–	–
Pyrimethamine (Pirimetamine)	11	Antimicrobial	Multiple (<5)	✔	✔	✔	–	–	–
Quinidine	99	Antiarrhythmic, antimicrobial	Multiple (≥10)	✔	✔	✔	✔	–	✔**
Quinine	95	Antimicrobial	Multiple (<10)	✔	–	–	✔	–	✔
Ranitidine	100	Antiulcerative	Multiple (<5)	✔	✔	✔	✔	–	–
Rifabutin	11	Antibacterial	Single (2003)	✔	✔	–	✔	–	–
Rifampin‡ (Rifampicin)	101–103	Antibacterial	Multiple (≥10)	✔	✔	✔	✔	–	✔**
Stibophen	105	Antimicrobial	Multiple (<5)	✔	✔	–	✔	–	–
Streptokinase	106	Thrombolytic	Single (1989)	✔	✔	✔	–	–	✔
Streptomycin	107–109	Antimicrobial	Multiple (<10)	✔	✔	✔	✔	–	✔
Sulfasalazine	110	Antiinflammatory	Multiple (<5)	✔	✔	–	✔	–	–
Sulfisoxazole	11	Antibacterial	Single (2003)	✔	✔	✔	✔	–	–
Sulindac	111	Antiinflammatory	Multiple (<10)	✔	✔	✔	✔	–	✔**
Suprofen	112	NSAID	Single (1989)	✔	✔	–	✔	–	✔**
Tartrazine	113	Colorant	Single (1979)	✔	✔	✔	✔	–	–
Teicoplanin	114	Antimicrobial	Single (2004)	✔	✔	–	✔	–	✔
Temafloxacin§	115	Antimicrobial	Multiple (<5)	✔	✔	–	✔	–	–
Teniposide	116	Antineoplastic	Single (1982)	✔	✔	–	✔	–	✔
Tetracycline	117	Antimicrobial	Multiple (<10)	✔	✔	✔	–	–	–
Thiopental sodium	104	Anesthetic	Single (1985)	✔	–	–	✔	–	–
Ticarcillin‡	118	Antimicrobial	Multiple (<5)	✔	✔	✔	–	–	✔
Tolbutamide	119	Antidiabetic	Multiple (<5)	✔	✔	✔	–	–	–
Tolmetin‡	120	NSAID	Multiple (≥10)	✔	✔	–	✔	–	✔**
Triamterene	121	Diuretic	Multiple (<5)	✔	✔	✔	✔	–	–
Trimellitic anhydride	122	Used in preparation of resins, dyes, adhesives, etc.	Single (1979)	✔	–	✔	–	–	–
Trimethoprim and sulfamethoxazole‡	123	Antibacterial	Multiple (<5)	✔	✔	✔	✔	–	✔
Vancomycin	11	Antibacterial	Single (2003)	✔	✔	–	✔	–	–
Zomepirac	124	NSAID	Single (1983)	✔	✔	–	✔	–	✔

* When a drug antibody is indicated to be reactive by two methods, e.g., vs. drug-treated RBCs and when serum + drug + RBCs are mixed together, this does not necessarily mean that all examples of antibodies to that drug were detected by both methods. Using ampicillin for example, four reported antibodies reacted with drug-treated RBCs and were either nonreactive (n = 1) or not tested (n = 3) by the serum + drug + RBCs method, and two antibodies reacted when serum + drug + RBCs were tested but were nonreactive with drug-treated RBCs.

IHA = immune hemolytic anemia; HA = hemolytic anemia; NSAID = nonsteroidal antiinflammatory drug.

† One or more samples only positive or strongest reactions seen with ex vivo (urine or serum) or metabolite.

‡ We have seen cases of DIIHA or positive DAT or both attributable to these.

§ No longer manufactured.

¶ Associated with nonimmunologic protein adsorption (NIPA).

**One or more samples positive possibly owing to the presence of circulating drug or drug–antibody immune complexes.

Table 2. Drugs associated with cases of IHA or positive DAT or both in which only drug-independent antibodies (autoantibodies) were detected

Drug (Alternative name)	Reference	Therapeutic category	Number of references [single (year) vs. multiple (<5, <10, ≥10)]	HA	Positive DAT	More evidence needed
Captopril	125	Antihypertensive	Multiple (<5)	✔	✔	✔
Chaparral	126	Herbal	Single (1980)		✔	✔
Cimetidine	127	Antiulcerative	Multiple (<10)	✔	✔	✔
Cladribine (2-chlorodeoxyadenosine)	128	Antineoplastic	Multiple (<10)	✔	✔	–
Fenfluramine	129	Anorexic	Single (1973)	✔	✔	✔
Fludarabine*	130,131	Antineoplastic	Multiple (≥10)	✔	✔	–
Interferon	132	Antineoplastic, antiviral	Multiple (≥10)	✔	✔	✔
Interleukin-2	133	Antineoplastic	Multiple (<5)	✔	✔	✔
Ketoconazole	134	Antifungal	Single (1987)	✔	✔	✔
Lenalidomide	135	Immunomodulatory	Single (2006)	✔	✔	✔
Levodopa (L-dopa)	136	Antiparkinsonian	Multiple (≥10)	✔	✔	–
Mefenamic acid	137	NSAID	Multiple (≥10)	✔	✔	–
Mesantoin (Mephenytoin)	138	Anticonvulsant	Single (1953)	✔	✔	✔
Methyldopa*	139	Antihypertensive	Multiple (≥10)	✔	✔	–
Nalidixic acid	140	Antibacterial	Multiple (<10)	✔	✔	✔
Procainamide*	141, 142	Antiarrhythmic	Multiple (<10)	✔	✔	–
Tacrolimus	143	Immunosuppressant	Multiple (<5)	✔	✔	✔

IHA = immune hemolytic anemia; HA = hemolytic anemia
* We have seen cases of DIIHA or positive DAT caused by these.

Table 3. Drugs associated with the detection of nonimmunologic protein adsorption onto RBCs

Drug (Alternative name)	Reference	Therapeutic category	Number of references [single (year) vs. multiple (<5, <10, ≥10)]	HA	Positive DAT	Drug-dependent antibody(ies) also detected
Cefotetan*	29, 30	Antimicrobial	Multiple (≥10)	✔	✔	✔
Cephalothin*	41, 42	Antimicrobial	Multiple (≥10)	✔	✔	✔
Cisplatin	51	Antineoplastic	Multiple (<10)	✔	✔	✔
Clavulanate potassium* (Clavulanic acid)	144, 145	β-Lactamase inhibitor	Multiple (<5)	–	✔	–
Diglycoaldehyde (INOX)	146, 147	Antineoplastic	Multiple (<5)	–	✔	–
Oxaliplatin*	91	Antineoplastic	Multiple (≥10)	✔	✔	✔
Sulbactam*	145, 148	β-Lactamase inhibitor	Multiple (<5)	✔	✔	–
Suramin	149	Antihelminthic, antiprotozoal	Single (1988)	–	–	–
Tazobactam*	150, 151	β-Lactamase inhibitor	Multiple (<5)	✔	✔	–

HA = hemolytic anemia
* We have seen cases of DIIHA or positive DAT caused by these.

drug-independent antibody (autoantibody). We should emphasize that the latter hypothesis above is our own and has little experimental data to support it. Alternative explanations are that these reactions, without the presence of drug, may be attributable to drug–immune complexes still present in the plasma, or enough drug present in the plasma to cause reactions with drug-dependent antibodies.[48,64,65,77,155–160] The reactions can be differentiated from true autoantibodies by repeating the testing a few days after the drug is discontinued; by then, the drug or drug complexes will no longer be present. If the drug has an unusually long half-life (e.g., mefloquine hydrochloride has a mean half-life of 3 weeks), or if the patient is in renal failure, then the circulating drug may persist longer than expected.

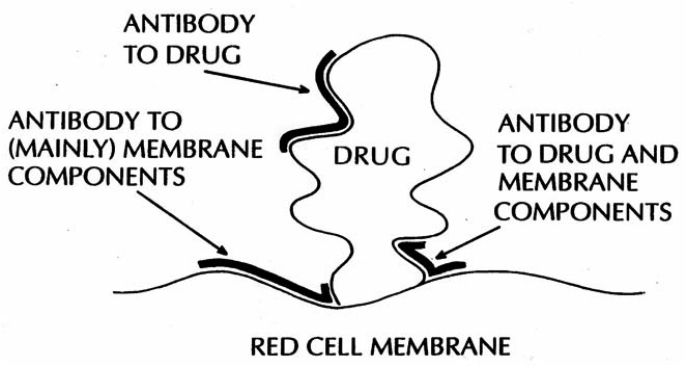

Fig. 1. Proposed unifying hypothesis of drug-induced antibody reactions. The thicker, darker lines represent antigen-binding sites on the Fab region of the drug-induced antibody. Drugs (haptens) bind loosely (or firmly) to RBC membranes, and antibodies can be made to the drug (producing in vitro reactions typical of a drug adsorption [penicillin-type] reaction); membrane components, or mainly membrane components (producing in vitro reactions typical of autoantibody); or part-drug, part-membrane components (producing an in vitro reaction typical of the so-called immune complex mechanism).

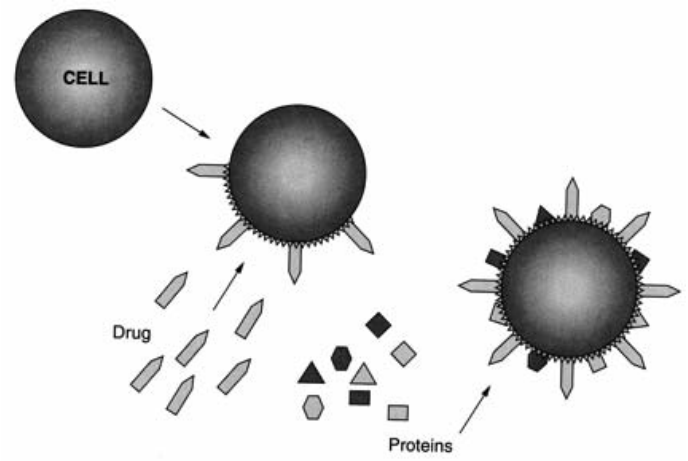

Fig. 2. Nonimmunologic adsorption of proteins onto RBCs. Drugs might change the RBC membrane so that many proteins attach to the membrane, leading to a positive DAT and, possibly, to DIIHA.

Table 2 is a list of 17 drugs that have been associated with drug-independent antibodies (autoantibodies). Many of these associations are not supported by very convincing proof that the drug induced the autoantibodies; idiopathic autoimmune hemolytic anemia (AIHA) is much more common than DIIHA and often is not excluded in the reports. It is not possible to prove the suggestion by laboratory testing as drug antibodies are not demonstrable, so many of the reports are based on a hemolytic anemia or a positive DAT or both developing after drug therapy and a response to stopping the drug. This could be coincidence. If it was a true phenomenon, then giving the drug again should restart the problem. For obvious reasons, this has not been tried often. It was tried, with methyldopa, and the AIHA occurred again, proving that there was a true relationship of drug and AIHA. Petz et al.[161] showed that it was a fallacy in two patients with suspected cimetidine-induced AIHA. When the patients' hemolytic anemia resolved after discontinuation of the drug, they were treated again with cimetidine (55 days in one patient and 24 months in the second patient) and no hemolytic anemia recurred, suggesting the drug had not caused the AIHA. These findings cast doubt on many of the drugs in this table. Often, when the drugs are stopped the patients are also taking steroids, so it is difficult to be certain what caused the recovery.

Positive Antiglobulin Tests Caused by Nonimmunologic Adsorption of Protein

During the last few years, we have become very interested in the nonimmunologic adsorption of protein onto RBCs leading to positive antiglobulin tests, and perhaps DIIHA. The reaction can mislead the investigator into thinking that an antibody to RBC antigens is being detected. The first drug shown to cause this phenomenon was the first cephalosporin (cephalothin). In 1971, Spath et al.[162] showed that cephalothin-treated RBCs adsorbed IgG, C3, albumin, fibrinogen, etc., after incubation in normal plasma; the proteins could be detected by the antiglobulin test. This is because the drug can modify the RBC membrane to adsorb proteins nonimmunologically (see Figure 2). Table 3 shows eight other drugs that cause the same effect. Up to about 1996, we believed that this was an in vitro phenomenon that caused us in vitro problems, but that it had no clinical significance. We now believe this mechanism can cause hemolytic anemia. RBCs having IgG on their membrane, caused by nonimmunologic adsorption, yield positive monocyte monolayer assays (MMA), and we have published data showing that the mechanism can be the probable cause of decreased RBC survival in patients taking drugs that contain β-lactamase inhibitors (clavulanate, sulbactam, tazobactam).[5,150,151]

This mechanism can lead to confusion in the workup of DIIHAs. The patient's serum may react with RBCs coated with the drugs listed in Table 3, but this means nothing as normal sera will also react with these same drug-coated RBCs. This is why it is important to use pooled normal plasma or serum as a negative control in any tests on drug-coated RBCs. Remember,

for five of these drugs, there is no drug antibody involved, so one can only suggest to the physician that this mechanism could be involved and let the physician have the relevant references.

Another complication is that drugs containing β-lactamase inhibitors also contain antibiotics (Zosyn contains tazobactam plus piperacillin; Unasyn contains sulbactam plus ampicillin; Timentin contains clavulanate plus ticarcillin; Augmentin contains clavulanate plus amoxicillin). These antibiotics can also cause DIIHA through more typical mechanisms.[5,163] Piperacillin is an exception to the typical reaction of penicillins in that it is detected best by the "immune complex" method.[7,164,165] An unusual aspect of piperacillin is that most normal donor and patient sera appear to have piperacillin antibodies as they react with piperacillin-coated RBCs, and this is not caused by nonimmunologic adsorption of protein. Normal sera also contain antibodies that react with penicillin- and cefotetan-coated RBCs,[5,166] but fewer sera react with these than with piperacillin-coated RBCs. It seems that we all are exposed to antigens identical, or very similar, to some penicillin-like structures in our environment.

A few technical hints when investigating DIIHA:

1. A positive DAT with a nonreactive eluate should not automatically suggest an investigation of DIIHA. Much more common causes are cytophilic IgG[167,168]; passively transfused anti-A or anti-B or both (e.g., when A, B, or AB patients receive group O RBCs, platelet products from donors not ABO identical, IVIG, or intravenous anti-D immunoglobulin).[169] Remember, drug-induced positive DATs and AIHA are rare.
2. Prepare penicillin-treated RBCs at high pH (e.g., 8–10), but prepare cephalosporin-treated RBCs at pH 7 to 7.4 to reduce nonimmunologic binding of protein.[5]
3. Some drugs do not fit the pattern seen with other members of the same family:
   - It is not possible to prepare ceftriaxone-coated RBCs; the "immune complex" method must be used.
   - Special conditions are needed to prepare nafcillin- and erythromycin-coated RBCs.[62,85,170,171]
   - Piperacillin-coated RBCs will be agglutinated by many normal sera; thus, piperacillin antibodies in serum should only be tested by the "immune complex" mechanism.[165] RBC eluates can be tested with piperacillin-coated RBCs.
4. Some drugs contain more than one chemical, e.g., Zosyn contains an antibiotic piperacillin and a β-lactamase inhibitor, tazobactam. It is important to test each drug separately to determine the specificity of the antibody. In addition to the problem with piperacillin (discussed in an earlier section), there is a special problem with the β-lactamase inhibitors. The patient's serum may react with drug-treated RBCs, suggesting a drug antibody; unfortunately, all normal sera will also react. This is because the drug can modify the RBC membrane to adsorb proteins nonimmunologically (see earlier section). Thus, it is essential to always use a pool of normal sera as a negative control when evaluating any DIIHA serologic results.
5. Antiglobulin test reactivity of a pooled normal sera control with drug-treated RBCs indicates the possible presence of nonimmunologic protein adsorption (NIPA).[5] If this occurs, the normal sera control and the patient's serum should be diluted 1 in 20 in saline and retested (the 1 in 20 dilutions should not contain enough protein to cause detectable NIPA). For testing of cefotetan-treated RBCs, the patient's serum should be diluted 1 in 100 because many normal sera react with cefotetan-treated RBCs, even at a 1 in 20 dilution.[166]
6. If a patient's serum contains a drug-independent antibody, the presence of a drug-dependent antibody can be demonstrated by performing an autologous or allogeneic adsorption to remove the drug-independent antibody and then testing the adsorbed serum by the usual methods to detect drug antibodies.
7. When looking at a patient's drug history, in addition to considering the drugs that the patient is receiving at the time of the hemolytic anemia, it may also be important to determine what drugs the patient may have received 1 to 2 weeks previously (e.g., in surgery).
8. It has been reported that 6% albumin can be used to solubilize drugs that have poor water solubility.[172] If the drug (e.g., cephalothin, cefotetan) bonds covalently to proteins (e.g., albumin), then reduced binding of the drug to RBCs can occur.[173] Thus, this method of

solubilizing drug in 6% albumin should be used cautiously, e.g., only when the drug is known to not be soluble in water and with the knowledge of its protein binding affinity (information about solubility and protein binding can usually be found in the Merck Index[174] or the Physicians' Desk Reference[175] or both).

9. Some drug antibodies have been reported to be detectable in vitro only when ex vivo preparations of the drug, e.g., urine or serum from a person taking the drug, have been used for the testing (by the "serum plus drug plus RBCs" method).[14,19,20,63,84,176–178] This is thought to be caused by the antibody being directed against a metabolite of the drug rather than the native drug and the presence of the appropriate drug metabolite(s) in the ex vivo preparation(s). Drug antibodies that have been reported to only be detected in the presence of an ex vivo preparation have been indicated with a footnote under the "serum plus drug plus RBCs" method in Table 1.
10. One important endpoint in testing for the presence of drug antibodies is hemolysis. A serum sample must be used for this testing (EDTA binds calcium, which is needed for the complement cascade; thus, EDTA plasma cannot be used for the detection of hemolysis). Many blood banks now use EDTA plasma for their routine testing, and the patient will have to have blood drawn again to obtain the necessary serum sample for a proper DIIHA workup.
11. If a patient's serum is nonreactive with drug-treated RBCs and there is no positive control available to confirm the presence of the drug on the drug-treated RBCs, then no interpretation of the negative result can be made.
12. When a drug antibody in the serum is detected by testing drug-treated RBCs, it is important to confirm the presence of that drug antibody in an eluate prepared from the patient's RBCs to conclude that the drug was the cause of the patient's hemolytic anemia.

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Review: Pharmacologic treatment of warm autoimmune hemolytic anemia

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The clinical course of warm autoimmune hemolytic anemia (WAIHA) can be perplexing and frustrating. Although many patients respond to standard therapy in a predictable and timely fashion, some patients are refractory to standard therapy and may require several attempts of therapies that are less well established. The focus of this review is to discuss the various pharmacologic approaches and options for the treatment of WAIHA.

Corticosteroids

Corticosteroids are the initial therapy of choice for WAIHA. A standard approach is to treat adults with prednisone, 1 to 1.5 mg/kg per day (or 60 to 100 mg/day) for 1 to 3 weeks. Clinical response with improvement in hematologic variables may be seen within several days to 1 week. Approximately 80 percent of patients have a good initial response to this therapy.[1–3] Most patients who are going to respond will respond within 2 weeks. If no response is noted within 3 weeks, steroid therapy has failed and alternative therapeutic options should be considered.

For patients who improve with corticosteroid therapy, the dosage of corticosteroids can be gradually reduced only after stabilization of hematologic variables. It is generally recommended to continue the initial higher dose of steroids for 1 to 2 weeks after achieving a response, weighing the benefits of continued steroid therapy against the risks of this therapy. After this period of stabilization, the steroid dose should gradually be tapered. Sudden decreases in dosage or rapidly progressive tapers can lead to relapse. If relapse does occur, the dose should be increased. Most clinicians consider a daily maintenance dose of prednisone greater than 15 mg to achieve a Hct of at least 30% a therapeutic failure, requiring other interventions.

The adverse effects of corticosteroid therapy are well established, and their severity should not be underestimated. Initial complications may include insomnia, weight gain associated with increased appetite, and emotional lability. Conditions such as diabetes and hypertension may present or worsen, if preexisting. Long-term corticosteroid therapy is complicated by the development of a cushingoid habitus, osteoporosis, and avascular necrosis. Ophthalmologic complications include posterior subcapsular cataracts and glaucoma. Patients are at increased risk of infection owing to steroid-related immunosuppression. The complications of steroid therapy can be quite severe; consequently, steroids must be used judiciously and doses should be tapered as quickly as possible.[2,4]

The explanation for clinical response to corticosteroids is likely multifactorial. Steroids have been shown to have an early effect on tissue macrophages, which become less efficient at clearing IgG- and C3-coated RBCs within the first 8 days of therapy.[5] Steroids may also affect antibody avidity.[6] Only after several weeks of therapy is there a significant decrease in antibody production.[6]

Permanent remission of WAIHA occurs in only approximately 20 to 35 percent of adult patients.[7,8] Consequently, additional therapy is generally planned because clinical relapse is likely.

Splenectomy

Although the focus of this review is to provide an overview of the pharmacologic options for the treatment of autoimmune hemolytic anemia (AIHA), it is difficult to discuss treatment options without mentioning the role of splenectomy. Splenectomy has traditionally been the second-line therapeutic approach, after corticosteroid therapy; this may be in transition as pharmacologic options are improving.

Approximately 50 percent of patients with WAIHA will have an excellent initial response to splenectomy, although low doses of prednisone (< 15 mg/day) may still be needed to maintain adequate hemoglobin levels.[9] Late relapses do occur, presumably as a result of enhanced antibody synthesis and increased hepatic sequestration.[1,8]

Although there is surgical morbidity and mortality associated with splenectomy, the most significant risk of adverse event related to splenectomy is overwhelming postsplenectomy sepsis syndrome. Infections with encapsulated bacteria represent a medical emergency because there may be rapid progression from an apparent flulike illness to bacteremic shock, with hypotension and disseminated intravascular coagulation. The risk of overwhelming postsplenectomy sepsis syndrome has been quantitated as 3.2 percent with a mortality rate of 1.4 percent.[10] The risks of both infection and mortality can be reduced by the use of pneumococcal and meningococcal vaccines. Prophylactic antibiotic regimens are controversial; however, many advocate the use of penicillin (250 mg twice a day); amoxicillin or Bactrim can be used as alternatives. Febrile illnesses in splenectomized patients must be given prompt attention and antibiotics administered expeditiously.

Because of this life-threatening risk associated with splenectomy and because of the increasing pharmacologic options, many clinicians are no longer routinely using splenectomy as a second approach after corticosteroid therapy.

Immunosuppressive Agents

Several immunosuppressive agents have been reported to be successful in the treatment of WAIHA, but predominantly in case reports and small series. In the past, these more intensive immunosuppressive regimens were only considered when there is lack of response to corticosteroids and splenectomy, when there is relapse after corticosteroids and splenectomy, when splenectomy is an unacceptable medical risk, or when corticosteroid therapy cannot be tolerated.

Azathioprine

Azathioprine, an immunosuppressive antimetabolite, is an imidazolyl derivative of 6-mercaptopurine. It is used for the prevention of renal allograft rejection, as well as the treatment of autoimmune disorders, such as rheumatoid arthritis (RA) and inflammatory bowel disease.

Azathioprine has been used with reported success in WAIHA. One study described 14 patients with idiopathic WAIHA who were treated with azathioprine; 6 of the patients (43%) achieved good response with normal hemoglobin levels.[11] In a report of 26 patients with AIHA in the setting of systemic lupus erythematosus (SLE), 2 patients received azathioprine for relapse after successful initial response to corticosteroids.[12] Both patients achieved chronic remission, and one of these patients was able to stop steroid therapy after initiating azathioprine.

The generally recommended dose of azathioprine for this indication is 1 to 2 mg/kg per day, or 75 to 200 mg/day in adults. If the patient is already taking steroids and has a partial remission, the steroids should be continued and tapered after a clinical response is achieved. If after 3 to 4 weeks the patient has not responded, the dosage may be increased (usually in increments of 25 mg/day); however, the adverse effects of azathioprine can be limiting.

The use of azathioprine is associated with gastrointestinal intolerance, including nausea, vomiting, and diarrhea, and dose-related bone marrow suppression with leukopenia and thrombocytopenia. Because azathioprine is cytotoxic, its prolonged administration is not advised because of the risk of development of a neoplasm.

Cyclosporine

This lipophilic cyclic protein binds to cytoplasmic proteins, called cyclophilins, and the resulting complex inhibits calcineurin. Consequently, cyclosporine inhibits selected cytokine transcription, down-regulating the transcription of some proinflammatory cytokines, and it also inhibits T-lymphocyte activation. Cyclosporine is used in the prophylaxis and treatment of solid-organ transplant rejection and in the management of several autoimmune disorders, including RA, ulcerative colitis, and psoriasis.

Cyclosporine has been used with reported success in the treatment of refractory WAIHA. Emilia and associates[13] described the successful use of cyclosporine in the treatment of three patients with AIHA and one patient with Evans' syndrome. All patients were refractory to multiple previous therapies including steroids, splenectomy, and immunosuppressive agents. The patients were treated at an initial total dose of 5 mg/kg per day given twice daily for 6 days with subsequent dose reduction to 3 mg/kg per day, maintaining a serum cyclosporine level between

200 and 400 ng/mL. Low-dose prednisone (5 mg/day) was given to increase cyclosporine blood concentrations. Dundar and colleagues[14] reported similar successful hematologic response in a patient with Evans' syndrome. The patient was refractory to standard dose and high-dose corticosteroid therapy and splenectomy, but responded to a cyclosporine regimen with an initial dose of 10 mg/kg per day gradually tapering to 4 mg/kg per day.

Others have successfully used cyclosporine in combination with corticosteroid therapy. Hershko and coworkers[15] presented three patients, two with AIHA and one with Evans' syndrome, who relapsed despite initial clinical response to steroids. All three patients showed clinical improvement with cyclosporine therapy (4 to 6 mg/kg per day) in addition to continued corticosteroid therapy. A child with refractory Evans' syndrome, who had failed corticosteroids and splenectomy, was successfully treated with cyclosporine and prednisone.[16] Initially, the cyclosporine was given at 10 mg/kg per day and prednisone, at 2 mg/kg per day. Each drug was gradually tapered, ultimately going to alternate day cyclosporine and prednisone dosing. One remarkable case is that of a 51-year-old woman with SLE who had AIHA that was refractory to steroids, splenectomy, cyclophosphamide, and azathioprine, but who responded to cyclosporine therapy, allowing for the corticosteroid tapering.[17]

Despite these reports of success, other authors have reported failure in treating AIHA with cyclosporine. Ferrara et al.[18] reported a 27-year-old man with AIHA in the setting of myelodysplastic syndrome (MDS). In addition to being refractory to cyclosporine, this patient did not respond to corticosteroids or immunoglobulin. He was successfully treated with a single high dose of cyclophosphamide (4 g/m^2).

The most common and significant adverse effect of cyclosporine therapy is nephrotoxicity. Although reversible acute azotemia can occur, irreversible progressive renal disease may also occur. Because of this significant risk of nephrotoxicity, patients taking cyclosporine must be monitored closely. Other adverse effects include hypertension, often related to renal vasoconstriction, gastrointestinal intolerance, and neurologic complications.

Mycophenolate Mofetil

After adsorption, mycophenolate mofetil is hydrolyzed to its active metabolite, mycophenolic acid, which has potent cytostatic effects on lymphocytes. It inhibits proliferation of T and B lymphocytes, and it suppresses antibody production. This immunosuppressive agent is routinely used with cyclosporine and corticosteroids for the prevention of renal, cardiac, and hepatic allograft rejection. It may also be used to treat psoriasis and proliferative lupus nephritis.

A few case reports suggest efficacy in the treatment of WAIHA. Howard et al.[19] reported treating four adult patients with AIHA with mycophenolate mofetil. All patients had failed previous therapy; two patients had been treated with prednisone, splenectomy, azathioprine, and cyclosporine, and two patients were previously treated with prednisone and cyclosporine. Mycophenolate mofetil was dosed as follows: 500 mg/day increasing to 1 g/day after 2 weeks. All four patients achieved a complete or good partial response to therapy. Kotb and colleagues[20] reported the use of mycophenolate mofetil in the treatment of 13 patients with autoimmune cytopenias, including 3 patients with AIHA and 1 patient with Evans' syndrome. The patients with AIHA were refractory to steroids, immunoglobulin, and cyclophosphamide. The same treatment protocol was used for all patients; an initial dose of mycophenolate mofetil 500 mg/day increasing to 1 to 3 g/day during the course of 1 to 2 weeks, depending on the patient's weight. Once therapeutic goals were reached, other associated treatments were tapered and stopped, followed by tapering of the mycophenolate mofetil. Within 4 to 6 months, all 3 patients with AIHA were independent of RBC transfusion. The patient with Evans' syndrome, who had been refractory to high-dose steroids and immunoglobulin therapy, responded within 6 weeks.

Mycophenolate mofetil has also been used to successfully treat AIHA in the setting of several underlying conditions. Zimmer-Molsberger and colleagues[21] treated two patients who had received 2-chlorodesoxyadenosine for underlying B-cell lymphocytic leukemia. Both patients had previously failed corticosteroid treatment. One patient achieved transfusion independence after mycophenolate mofetil therapy. The other patient had a partial response but was able to decrease his RBC requirement by more than half. In the setting of MDS, Lin et al.[22] reported the successful use of mycophenolate mofetil. The patient had failed corticosteroid therapy alone. Although cyclosporine was tried, it was discontinued owing to neurotoxicity. After starting mycophenolate therapy at 1 g/day with prednisolone (15 mg/day), prednisolone was tapered and stopped within the following 3

weeks. Four weeks after the initiation of mycophenolate mofetil, the patient was transfusion independent. Alba and colleagues[23] described the successful use of mycophenolate in the treatment of two patients with AIHA in the setting of SLE and antiphospholipid syndrome. Both patients were given mycophenolate mofetil (1 to 2 g/day) for the treatment of lupus nephritis, but the authors noted an improvement in hematologic variables temporally associated with the mycophenolate mofetil therapy.

The adverse effects of mycophenolate mofetil tend not to be as severe compared with other immunosuppressive drugs. Some patients may experience gastrointestinal intolerance, and myelosuppression may be associated with this drug.

Cyclophosphamide

Cyclophosphamide is a cytotoxic, alkylating agent that is rapidly absorbed and converted by the liver to its active metabolite. It impairs DNA replication and transcription, ultimately resulting in cell death. All metabolites of the drug are excreted in the urine. The degree of immunosuppression and cytotoxic effects are related to the dose and duration of treatment.

Cyclophosphamide has been used in a variety of dose regimens for the treatment of AIHA. One suggested dosage is 1.5 to 2 mg/kg per day. If the patient is already taking corticosteroids, the steroids should be continued. If there is no hematologic improvement after 4 weeks, the dose can be increased in increments of 25 mg/day every 2 weeks.

Cyclophosphamide was successful in the treatment of a 12-year-old girl with AIHA in the setting of giant cell hepatitis.[24] Although the etiology of giant cell hepatitis has not been entirely elucidated, an immunologic pathogenesis has been proposed. This patient failed conventional dose and high-dose prednisone, azathioprine, and IVIG. After the addition of cyclophosphamide at a dose of 1.5 mg/kg per day to her baseline prednisone and azathioprine, the patient experienced resolution of both hematologic and hepatic variables.

A report by Panceri et al.[25] described a 5-month-old boy who had life-threatening AIHA. This child was refractory to steroids, high-dose immunoglobulin, azathioprine, and splenectomy. The patient required intensive transfusion support, receiving two to three RBC transfusions per day. Because of the severity of the clinical situation, the child was given high-dose methylprednisolone (40 mg/kg per day) followed by high-dose cyclophosphamide (10 mg/kg per day for 10 days). The child experienced striking, sudden improvement, ultimately achieving complete recovery without any major long-term complications.

Ferrara and colleagues[18] described the successful treatment of a 27-year-old man with refractory AIHA in the setting of refractory anemia, a subtype of MDS. The patient had failed the following treatments: high-dose methylprednisolone, high-dose immunoglobulin, and cyclosporine. The patient was treated with a single, high dose of cyclophosphamide (4 g/m^2) followed by daily filgrastim in an effort to mobilize CD34+ cells. On days 12 and 13, apheresis was performed to harvest peripheral stem cells in anticipation of an autologous peripheral stem cell transplant. The patient's hematologic counts recovered, and at 11 months' follow-up, his counts continued to be normal and he did not require a stem cell transplant.

High-dose cyclophosphamide without stem cell rescue was purposefully used by Moyo et al.[26] They report a series of nine patients with severe refractory hemolytic anemia. All patients had failed a median of three prior treatments (range, 1 to 7). Patients received cyclophosphamide 50 mg/kg per day for 4 days followed on day 6 by daily granulocyte colony-stimulating factor (5 μg/kg). This therapy successfully reversed refractory disease, achieving complete remission in six patients and partial remission in three patients of the nine treated. These investigators have subsequently reported successful use of this regimen for the treatment of other refractory autoimmune diseases, including SLE,[27] myasthenia gravis,[28] severe aplastic anemia,[29] and hepatitis-associated aplastic anemia.[30]

The severity of the adverse effects related to cyclophosphamide is dependent on the dose and duration of therapy. The toxicities include bone marrow suppression, increased susceptibility to infection, infertility as a result of gonadal toxicity, risk of malignancy, and bladder toxicities including cystitis and risk of bladder cancer. When high-dose cyclophosphamide is used, it is recommended to also give mesna to prevent hemorrhagic cystitis. High-dose regimens are also associated with nausea, alopecia, and cardiac toxicity.

Danazol

Danazol is a semisynthetic, attenuated androgen that was initially used for the treatment of endometriosis. Subsequently, it was found to be effective in the

treatment of fibrocystic breast disease and hereditary angioedema. Danazol has been helpful in a few cases of WAIHA. Its mechanism of action is uncertain in this clinical setting, although it has been suggested that it is an immunomodulatory drug that may decrease IgG production and reduce RBC-bound IgG and complement.

In the largest report, Ahn[31] described 28 patients with AIHA who were treated with prednisone 20 to 60 mg/day and danazol 600 mg/day. Once the hemolysis stopped, prednisone was tapered and ultimately discontinued. Of the 13 patients with idiopathic AIHA, 77 percent of patients had an excellent or good response. Fifteen of the patients had secondary AIHA caused by an underlying condition, including 12 patients with SLE. Sixty percent of the patients with secondary AIHA had an excellent or good response. The author noted that the side effects of danazol therapy were less than those of the steroids.

Pignon et al.[32] reported on the use of danazol in 17 adults with AIHA. Ten patients were newly diagnosed, and 7 patients were refractory to multiple therapies or had relapsed after initial steroid therapy. Patients were treated with prednisone (1 mg/kg per day) and danazol (600 to 800 mg). Once hemolysis was controlled, the prednisone was tapered or stopped. Long-lasting responses were noted in 80 percent of the newly diagnosed patients and in 60 percent of the previously treated patients. Only minimal side effects occurred.

Chan and Sack[33] reported a successful response to danazol in one patient with SLE and severe AIHA. This patient had been refractory to numerous therapies, including corticosteroids, splenectomy, azathioprine, chlorambucil, and IVIG.

In a series of 16 consecutive patients with SLE and AIHA or Evans' syndrome, danazol was given at an initial dose of 200 mg/day and was increased stepwise to a maximum dose of 1200 mg/day.[34] All 16 patients achieved a complete remission within 2 months after starting danazol. Most patients tolerated the drug well; however, some patients had undesirable side effects including weight gain, dizziness, rash, hepatic adenoma, cholestatic hepatitis, and pseudotumor cerebri.

Adverse effects include androgenic effects such as acne, hair loss, hirsutism, and amenorrhea. More severe effects also may be seen. Hepatic effects of danazol include increased transaminases, cholestatic jaundice, and hepatic adenoma. Changes in lipids may occur with increased risk of atherosclerosis. There is also an increased risk of thromboembolism and thrombotic complications.

Antibody Preparations

Intravenous Immunoglobulin

IVIG is manufactured from the pooled plasma of healthy donors. After a fractionation process, the product consists primarily of concentrated immunoglobulin, largely IgG. It is well established as an effective treatment for immune thrombocytopenic purpura (ITP). Despite its efficacy in a seemingly related disease, IVIG has not been shown to have comparable efficacy in WAIHA. IVIG is recommended for the treatment of AIHA only when patients are refractory or cannot tolerate first-line therapy. In a recent review of the use of IVIG in a large tertiary hospital, a total of 194 patients were treated with IVIG in 2004; only 6 of these patients (3%) were treated for AIHA.[35]

One study reported 37 patients in combination with 36 patients from the literature; all 73 patients had AIHA and were treated with IVIG.[36] Overall, 29 of 73 patients (39.7%) responded to IVIG therapy. The patients who responded were more likely to have hepatomegaly (with and without splenomegaly) and low initial hemoglobin. The authors suggest that IVIG is not optimal as standard therapy for AIHA, but has a role as adjunctive therapy especially for patients with low initial hemoglobin or hepatomegaly, or for those patients who cannot tolerate the toxicities of standard therapy.

A subsequent case report described a man with refractory and life-threatening AIHA in the setting of primary antiphospholipid syndrome.[37] He ultimately responded to a 5-day course of IVIG at a dose of 400 mg/kg per day. At the completion of the initial course of IVIG, hemolysis recurred and failed to respond to subsequent steroids, azathioprine, and cyclosporine. A second course of IVIG was successful in controlling his hemolysis followed by weekly maintenance of 800 mg/kg IVIG.

The adverse effects of IVIG are predominantly related to reactions occurring during infusion. Many of these reactions, which are generally self-limited, can be avoided by using a slower infusion rate. Consequently, it is usually recommended to infuse the initial dose at a slow rate, and, if well tolerated, the rate of infusion can be increased for subsequent doses. Aside from infusion-related adverse effects, the side effects of IVIG therapy are usually well tolerated.

Rituximab

Rituximab is a genetically engineered chimeric murine/human monoclonal anti-CD20 antibody that targets B-cell precursors and mature B cells; plasma cells do not carry the CD20 antigen. Rituximab is approved for the treatment of B-cell non-Hodgkin's lymphoma and B-cell chronic lymphocytic leukemia (CLL). Surprisingly, success of rituximab has not been limited to WAIHA secondary to B-cell neoplasms. The typical dosing regimen of rituximab for the treatment of WAIHA is 375 mg/m^2, weekly for 2 to 4 weeks, with some patients being treated for up to 12 weeks.[38]

Children with idiopathic WAIHA have responded to rituximab therapy. Quartier and colleagues[39] treated five children with refractory idiopathic AIHA and one child with AIHA after bone marrow transplantation. All children were refractory to prednisone and other therapies. The children ranged in age from 7 to 35 months. All patients achieved complete remission and remained in remission with 15 to 22 months' follow-up. Of note, patients experienced prolonged absence of B cells and hypogammaglobulinemia, such that five patients received prophylactic IVIG replacement for 9 to 10 months after completing rituximab therapy.

Zecca et al.[40] prospectively treated 15 children with refractory AIHA. All patients had previously failed two or more immunosuppressive therapies, and two of the children had undergone splenectomy. Four of the patients had underlying clinical conditions, including SLE, RA, vitiligo, and prior bone marrow transplantation. After completion of rituximab therapy, all patients received IVIG for 6 months. With a median follow-up of 13 months, 87 percent of patients (13 of 15) responded; 2 patients did not respond. Of the 13 patients who initially responded, 3 patients relapsed 7 to 10 months after therapy; all 3 patients responded to a second course of rituximab therapy.

Numerous single case reports and small series of adult patients report the successful use of rituximab in the treatment of refractory AIHA. Ahrens and colleagues[41] report a 68-year-old man with refractory disease who had failed previous therapies including steroids, azathioprine, cyclophosphamide, and mycophenolate mofetil. The patient experienced minimal side effects with chills associated with the first infusion. The patient's hemoglobin increased to 12.3 g/dL, and he became asymptomatic.

One of the larger series is reported by D'Arena and coauthors.[42] They report 11 adult patients with idiopathic WAIHA. Their retrospective analysis includes refractory patients who had failed corticosteroids, azathioprine, and high-dose immunoglobulins. At a mean follow-up of 604 days, 8 patients (73%) had achieved complete remission, and 3 patients (27%) had a partial remission. All patients were transfusion independent. The authors support the use of rituximab for steroid-refractory disease.

Shanafelt and colleagues[43] retrospectively reviewed the experience of five patients with AIHA and four patients with Evans' syndrome. Complete response occurred in two of the five patients (40%) with refractory AIHA. One patient with Evans' syndrome had resolution of ITP, and one patient had a complete response in AIHA; none of the patients with Evans' syndrome had resolution of both.

In the setting of CLL, rituximab has been successful in the treatment of AIHA. Narat et al.[44] presented 11 patients with chronic WAIHA refractory to numerous prior therapies. Of the 11 patients, 4 had underlying CLL and 1 patient had Waldenström's macroglobulinemia. Seven of the 11 patients (63.6%) responded to rituximab therapy, with 3 patients in complete remission and 4 patients in partial remission. The authors noted that the median duration of response was 11 months (range, 2.5 to 20 months).

D'Arena and coauthors[45] reported 14 patients with AIHA with underlying CLL. Three patients did not complete the full course of four doses; 2 patients died and 1 HCV-positive patient experienced a rise in amino transferases. An increase in hemoglobin was seen in all but 2 patients after rituximab therapy. Three patients (22%) were considered to have a full response, and 7 patients (50%) had a partial response.

The adverse effects of rituximab include infusion-related reactions, which may be quite severe. Patients may experience fevers, chills, and rigors, and in more severe cases, hypotension and even bronchospasm. The drug is associated with prolonged B-cell depletion, and, consequently, the risk of infection is long-lasting.

Alemtuzumab

Alemtuzumab, or Campath-1H, is a humanized rat IgG1 monoclonal antibody directed against CD52, which binds the cell membrane of lymphocytes, both B cells and T cells. This drug can induce a prolonged lymphopenia leading to extensive and long-lasting immunosuppression. The drug is used in the treatment of CLL, and it has been incorporated into stem cell transplant regimens.

There are far fewer reports on the use of alemtuzumab for the treatment of AIHA, most likely reflecting a relative lack of experience with this drug. Willis and colleagues[46] report 21 patients with refractory autoimmune cytopenias, including 2 patients with WAIHA and 3 patients with Evans' syndrome. Campath-1H was given as a 10-mg daily dose for 10 days. The patients with WAIHA both responded to therapy, one with a complete response and the other with a partial response. Two of the patients with Evans' syndrome responded, but both subsequently relapsed.

In a particularly dramatic case, a 58-year-old man with refractory AIHA failed numerous therapies, including steroids, azathioprine, splenectomy, and even rituximab.[47] This patient successfully responded after a regimen of alemtuzumab of 3 mg on day 1, 10 mg on day 3, and 30 mg on day 5, followed by 30 mg three times per week for 8 weeks. After this regimen, the patient's transfusion requirements decreased dramatically, and the alemtuzumab was gradually tapered. The patient experienced infusion-related chills and reactivation of CMV, requiring ganciclovir treatment.

Several small reports discuss the successful treatment of refractory AIHA in the setting of CLL. Karlsson and colleagues[48] reported five patients with B-cell CLL complicated by AIHA. The patients were transfusion dependent and refractory to previous therapy for AIHA including steroids and, in select patients, immunoglobulins, cyclosporine, cyclophosphamide, and rituximab in two patients. Patients were treated with an initial dose of 3 mg or 10 mg of alemtuzumab administered either subcutaneously or as an intravenous infusion. The doses were gradually increased to 30 mg, given three times per week for 12 weeks. All five patients responded with an increase in hemoglobin of more than 2 g/dL, no longer requiring transfusion support. At the end of treatment, the mean hemoglobin was 11.9 g/dL, and after 12 months' follow-up, the mean hemoglobin was 12.5 mg/dL.

The side effects related to alemtuzumab are predominantly infusion related, including fevers and chills. The most significant adverse effect of the treatment is prolonged lymphopenia and immunosuppression.

Eculizumab

This humanized monoclonal antibody is directed against the terminal complement protein C5. The antibody prevents cleavage of C5 into its proinflammatory components, inhibiting terminal complement activation. The drug has recently been approved for use in patients with paroxysmal nocturnal hemoglobinuria (PNH). In the initial study, 11 patients with PNH were treated with eculizumab using a regimen of 600 mg/week for 4 weeks, followed by 900 mg/week every other week through week 12. This study found eculizumab to be effective in reducing intravascular hemolysis, hemoglobinuria, and the need for transfusion in patients with PNH.[49] In an extension of the initial trial, the authors evaluated the long-term safety and response of eculizumab in the same 11 patients; the drug was found to have continued long-term efficacy and safety.[50] In a subsequent double-blind, multicenter trial, 87 patients were randomized to receive either placebo or eculizumab. This definitive trial confirmed the prior findings, with eculizumab reducing intravascular hemolysis and transfusion requirements with improvement in quality of life for patients with PNH.[51]

Although there are no reports to date in which eculizumab has been used in the treatment of AIHA, one must ask whether this drug would be useful particularly in refractory, life-threatening cases in which intravascular hemolysis is a key feature. In most cases of WAIHA, the hemolytic process is predominantly extravascular. But we have all been haunted by the unusual, troublesome patient who has a significant component of intravascular hemolysis. Perhaps it is in this setting of uncontrollable, refractory, and life-threatening intravascular hemolysis that eculizumab, or another complement inhibitory drug, may play a role in stabilizing the intravascular hemolysis, allowing the time for other therapeutics to be effective.

Conclusion

In an attempt to focus on the pharmacologic treatments of WAIHA, this review deliberately omits the role of transfusion therapy. Appropriate transfusion management is critical in the treatment of patients with life-threatening anemia, particularly those with a brisk hemolytic rate or reticulocytopenia. Transfusions may be needed for initial stabilization and should be anticipated during the patient's clinical course, such that appropriate serologic evaluations can be completed and optimal RBCs can be selected.

The therapeutic options for treating WAIHA are increasing with new immunosuppressive agents, monoclonal antibody preparations, and potentially complement inhibitory drugs. Despite these increasing therapeutic options, recommended initial

treatment remains corticosteroid therapy followed by a second-line approach of splenectomy. Because of the invasive nature of splenectomy and the lifelong risk of overwhelming postsplenectomy sepsis syndrome, in practice many clinicians are currently opting for pharmacologic agents, especially rituximab, as a second-line approach instead of splenectomy. One must be well aware that there are few definitive trials to support the use of these pharmacologic therapies as a second-line approach. The majority of the supportive evidence is based on case reports and small series of patients.

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Anti-P1: don't miss the obvious

R.J. ACKLEY, K.M. BYRNE, AND P.E. WEDDINGTON

Clinical Case Presentation

A sample collected from a 63-year-old Caucasian woman who donated platelets by apheresis was received for resolution of an ABO typing discrepancy. The donor had donated eight times during the past 16 years and always typed as group A, D+. No ABO typing discrepancies had ever been noted.

Immunohematologic Evaluation and Results

Forward and reverse typing was initially performed using the gel method for ABD typing (A/B/D Monoclonal and Reverse Grouping Card with A_1 and B reagent RBCs, Ortho-Clinical Diagnostics, Raritan, NJ). Plasma was tested for unexpected antibodies using pooled reagent RBCs and the IgG-gel test (ID-MTS Gel Test, Ortho-Clinical Diagnostics). Both gel cards were read by an automated reader (SA Reader, Ortho-Clinical Diagnostics). Because the sample's forward and reverse results did not correlate, the card was marked to be read manually. Agglutination (4+) was noted with anti-A; no agglutination was noted with anti-B. Conversely, agglutination was noted with both A_1 and B reagent RBCs, 2+ and 4 +, respectively. The Rh type was D+. The antibody screen with the pooled RBCs was negative.

ABO and Rh determination was repeated using the tube method. Unexpected plasma reactivity was again noted with A_1 reagent RBCs (Table 1). This serologic picture is one that can be observed in a donor or patient with an A_2 phenotype who has produced anti-A_1. To investigate this possibility, the RBCs were tested with A_1 lectin (Immucor, Inc, Norcross, GA). The RBCs typed positive for A_1, having the same strength as A_1 control RBCs. Because the donor was determined to be of the A_1 phenotype, the possibility that the unexpected reactivity noted in the reverse typing was attributable to anti-A_1 was ruled out.

Another possible explanation for the ABO typing discrepancy could be the presence of an alloantibody that reacts at room temperature and the A_1 reverse reagent RBCs being positive for the corresponding antigen. The fact that the initial antibody screen using pooled RBCs tested in the IgG-gel card was negative suggested that this was not the case. However, to further investigate this possibility, donor plasma was tested with a three-cell screen panel (Surgiscreen, Ortho-Clinical Diagnostics) using both IgG-gel card (ID-MTS Gel Test, Ortho Clinical Diagnostics) and LISS (ImmuAdd, ImmucorGamma, Norcross GA) IgG-IAT including an autocontrol. Tube testing included a 5-minute room temperature incubation. Agglutination was observed only by the tube method with screening cell I, which was identified as having a strong expression of P1 (Table 2). An additional P1+ (strong) reagent RBC was tested with the donor's plasma and again agglutination was noted after a 5-minute room temperature incubation. Anti-P1 was identified in the plasma because two reagent RBCs positive for P1 gave the expected positive results and two reagent RBCs negative for P1 gave the expected negative results and there was no unexplained reactivity. All other antibodies were ruled out. The RBCs of the platelet donor typed as P1–.

Before the workup could be considered complete, the unexpected reactivity with A_1 reverse reagent RBCs required resolution. First, to confirm that the reactivity noted with these RBCs was caused by the presence of anti-P1 in the plasma, they were typed and found to be P1+. Next, A_1 RBCs that were P1– were needed to

Table 1. ABO/D determination

	Donor RBCs with				Donor plasma with		
Method of determination	**Anti-A**	**Anti-B**	**Anti-D**	**Rh control**	**A_1 RBCs**	**B RBCs**	**A_2 RBCs**
A/B/D Monoclonal and Reverse Grouping Card	4+	0	4+	0	2+	4+	NT
Tube method	4+	0	4+	0	1+	4+	0

NT = not tested

Table 2. Results of antibody screen

				LISS			Gel
Cell	**Rh**	**P1**	**5-min RT**	**37°C**	**IAT-IgG**	**Check cells**	**IAT-IgG**
SC 1	R_1wR_1	+s	1+	0	weak	NT	0
SC 2	R_2R_2	+	0	0	0	+	0
SC 3	rr	0	0	0	0	+	0
Autocontrol			0	0	0	+	0

RT = room temperature; SC = screening cell; NT = not tested

repeat the reverse typing. Ten group A donor RBCs were typed for P1; one was P1– (Anti-P_1 Murine Monoclonal, Gamma-Clone, Gamma Biologicals, Inc, Houston, TX). This RBC was then typed with A_1 lectin and was positive. This A_1+, P1– RBC was then used to perform ABO reverse typing with the donor's plasma. No agglutination was noted. This concluded all testing necessary to resolve this ABO typing discrepancy. U.S. Food and Drug Administration (FDA)–licensed reagents should be used when resolving ABO discrepancies. In this case, donor group A RBCs were used in the resolution, and FDA-licensed reagents were used to type the selected RBC as A_1+, P1–.

Interpretation

This case describes the resolution of an ABO typing discrepancy caused by anti-P1. The antibody was detected in the plasma when unexpected agglutination was observed with A_1 reagent RBCs in the reverse typing. Anti-A_1 was ruled out after confirming the donor RBCs tested as group A_1. Anti-P1 was demonstrable in the tube after a 5-minute room temperature incubation with RBCs that were noted to strongly express P1. Anti-P1 was not detected by IgG-gel card.

Recommended Therapy

Testing to detect unexpected clinically significant antibodies to RBC antigens for allogeneic donors is required to be performed on serum or plasma from donors with a history of transfusion or pregnancy.[1] RBC components containing such antibodies that are not removed by being washed or deglycerolized must be labeled with the specificity of the unexpected RBC antibody.[1] Generally, blood components containing unexpected antibodies are made because the status of donor testing is not known at the time of component preparation. Eligibility for transfusion and possible modification of blood components containing unexpected clinically significant antibodies are determined by the transfusion facility's standard operating procedures. Plasma made into FFP is usually discarded. RBC or platelet apheresis components can be washed to remove plasma. Washing would remove the unexpected antibody, but the expiration of these components would be significantly shortened and the cost of the component would increase because of the supplies and labor needed to perform the task. Alternatively, these components can be given to patients whose RBCs are negative for the identified corresponding antigen. This involves typing patient samples and good inventory management. Yet another option to handle components with unexpected antibodies is to transfuse them without regard to antibody specificity. In a study by Coombs, Bennett, and Telen it was concluded that RBC units containing alloantibodies can be safely transfused to patients.[2] However, there are two major concerns associated with the transfusion of components containing non-ABO antibodies. One concern is that passively transfused antibody will be detected in posttransfusion blood samples. The second concern is that hemolysis may occur if the patient expresses the antigen corresponding to the passively acquired antibody. These concerns were noted in the Coombs study; however, no harm to patients was reported and a cost-saving was estimated. In this case, the platelet component was accidentally vented when a sample was being taken for bacterial testing, thus requiring the component to be discarded. If the component had been successfully sampled and determined to be negative for bacterial contamination, it would have been labeled as containing anti-P1. For transfusion purposes, the component would have been washed before issue. If the donor appeared later and no ABO typing discrepancy was noted and the antibody screen was negative, the component would not receive special labeling nor would it be washed before issue.

Discussion

Anti-P1 was identified in donor plasma and was the cause of the ABO typing discrepancy. This antibody is common in sera of P1– individuals, yet often goes undetected because it reacts optimally at 4°C.[3] The antibody is not typically considered clinically significant. The strength of expression of the P1 varies among individuals.[3,4] This fact presents a confusing and

often challenging picture when trying to identify an anti-P1 in the sera of such individuals. It was the ABO typing discrepancy that prompted an antibody investigation in this case, and it was P1+ (strong) RBCs identified by reagent manufacturer's testing that aided in the identification of the anti-P1. Because reactivity was only noted with RBCs that were identified as having a strong expression of P1, the antibody identity was obvious. If the reagent RBC was not marked as P1 strong, the identification may have been more challenging. If one suspects an antibody that would show reactivity at room temperature, then RBCs known to carry antigens such as M, N, Le^a, and Le^b should be selected. RBCs known to have a stronger expression of P1 should also be considered. For example, RBCs from African Americans (R_0 or V+) would be good choices for a selected RBC panel. Inhibition of blood group antibodies by soluble antigens is a method that could have been applied in this case to prove or disprove the presence of anti-P1.

ABO typing discrepancies can occur in either the forward or the reverse grouping, yet most will be attributed to an aberrant reverse typing.[5] Commercial ABO grouping reagents usually produce strong agglutinations, so it is often the weaker reaction that is suspect. In our case, the reverse typing was suspect when the weak reaction was noted with A_1 reagent RBCs. On review of the case, a more efficient workup would have been to perform the room temperature incubation with reagent screening RBCs and an autocontrol after confirming that the donor was group A_1. Testing the plasma in gel cards with the three reagent screening RBCs did not help to resolve the discrepancy other than to demonstrate that the gel method is ideal for avoiding clinically insignificant cold agglutinins. In the current environment in which the mantra is "work smarter not harder," it is important to evaluate all initial serologic reactions carefully, then decide the most logical and efficient next step. Let's not miss the obvious!

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Ricci J. Ackley, BS, MT(ASCP), Karen M. Byrne, MDE, MT(ASCP)SBB, CQA(ASQ), (corresponding author) and Patricia E. Weddington, MT(ASCP)CM, National Institutes of Health, Clinical Center, Department of Transfusion Medicine, Building 10, Room 1C711, 10 Center Drive MSC 1184, Bethesda, MD 20892-1184.

Monoclonal antibodies available at no charge:

The New York Blood Center has developed a wide range of monoclonal antibodies (both murine and humanized) that are useful for donor screening and for typing RBCs with a positive DAT. These include anti-A_1, -M, -s, -U, -D, -Rh17, -K, -k, -Kp^a, -Js^b, -Fy^a, -Fy3, -Fy6, -Wr^b, -Xg^a, -CD99, -Do^b, -H, -Ge2, -Ge3, -CD55 (both SCR2/3 and SCR4), -Ok^a, -I, and anti-CD59. Most of the antibodies are murine IgG and require the use of anti-mouse IgG for detection (Anti-K, -k, and -Kp^a). Some are directly agglutinating (Anti-A_1, -M, -Wr^b and -Rh17) and a few have been humanized into the IgM isoform (Anti-Js^b). The antibodies are available at no charge to anyone who requests them. Please visit our Web site for a complete list of available monoclonal antibodies and the procedure for obtaining them.

For additional information, contact: Gregory Halverson, New York Blood Center, 310 East 67th Street, New York, NY 10021 / e-mail: ghalverson@nybloodcenter.org (phone 212-570-3026, FAX: 212-737-4935) or visit the Web site at http://www.nybloodcenter.org >research >immunochemistry >current list of monoclonal antibodies available.

Meetings!

April 11–13 American Red Cross Immunohematology Reference Laboratory (IRL) Conference 2008

The American Red Cross Immunohematology Reference Laboratory (IRL) Conference 2008 will be held April 11 through 13, 2008, at the Chaparral Suites Resort, in Scottsdale, Arizona. For more information, contact Cindy Flickinger at (215) 451-4909 or flickingerc@usa.redcross.org.

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4. Acknowledgments
5. References
6. Author information
7. Tables
8. Figures

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