Immunohematology

JOURNAL OF BLOOD GROUP SEROLOGY AND EDUCATION

VOLUME 21, NUMBER 2, 2005

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CONTENTS

Immunohematology is published quarterly (March, June, September, and December) by the American Red Cross, National Headquarters, Washington, DC 20006.

Immunohematology is indexed and included in *Index Medicus* and MEDLINE on the MEDLARS system. The contents are also cited in the EBASE/Excerpta Medica and Elsevier BIOBASE/Current Awareness in Biological Sciences (CABS) databases.

The subscription price is $30.00 (U.S.) and $35.00 (foreign) per year.

Subscriptions, Change of Address, and Extra Copies:
Immunohematology, P.O. Box 40325
Philadelphia, PA 19106
Or call (215) 451-4902
Web site: www.redcross.org/pubs/immuno

ISSN 0894-203X

Review: Cromer and DAF: role in health and disease

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The antigens of the Cromer blood group system are located on the protein decay-accelerating factor (DAF). This system consists of ten high-prevalence and three low-prevalence antigens; the molecular basis for all of these antigens is a single nucleotide polymorphism in the *DAF* gene. DAF is a 70,000-Da plasma membrane protein that is widely distributed on all blood cells and on endothelial and epithelial tissues. The physiological role of DAF is to inhibit the complement cascade at the level of the critical C3 convertase step. By this mechanism, DAF acts to protect autologous cells and tissues from complement-mediated damage and hence can play a role in preventing or modulating autoimmune disease and inflammation. The use of recombinant DAF as a therapeutic agent in autoimmunity and inflammation, and of DAF transgenic animals in xenotransplantation, is being actively investigated. Additionally, DAF serves as a receptor for certain strains of *Escherichia coli* and certain types of enteroviruses. The DAF protein that contains the Cromer antigens serves important roles in health and disease. ***Immunohematology* 2005;21:39–47.**

Key Words: Cromer blood group system, decay-accelerating factor, DAF, CD55, complement

Introduction and Historical Background

The first antigen of what would become the Cromer blood group system was identified in 1965, based on an antibody found in the serum of a Black prenatal patient named Mrs. Cromer.[1] Four additional examples of antibodies to this high-prevalence antigen, Cr^a, were reported in 1975[2] and the null phenotype, Inab, was identified in 1982.[3] A total of ten high-prevalence antigens and three low-prevalence antigens have now been described in the Cromer blood group system. Since publication 3 years ago of a very comprehensive review of the serology, biochemistry, and molecular biology of the Cromer blood group system in *Immunohematology,*[4] two additional antigens, SERF[5] and ZENA,[6] have been described. Furthermore, two articles in this issue of *Immunohematology* continue the work on the Cromer blood group system, reporting the molecular basis for a new proband with the Inab phenotype due to a nonsense mutation[7] and measuring the *SERF* allele frequency at 1.1 percent in the Thai blood donor population.[8]

While this work on describing and characterizing the Cromer blood group system was proceeding, research from an independent direction resulted in the identification in 1969 of a substance in the plasma membrane of the RBC that acted as an inhibitor of the complement system.[9,10] In 1981, this 70,000-Da protein was purified and named decay-accelerating factor (DAF, CD55) because of its ability to accelerate the decay of the C3 convertase enzyme complex.[11,12] Over the subsequent years, extensive research has elucidated the structural and functional features of DAF, including its attachment to the cell membrane through a glycosylphosphatidylinositol (GPI) anchor[13,14] and the cloning and sequencing of *DAF* cDNA.[15,16]

These two independent lines of research came together in 1988 with the demonstration that the Cromer blood group antigens reside on the DAF protein.[17,18] This permitted the subsequent full molecular characterization of the Cromer blood group system. The reader is referred to the recent review in *Immunohematology*[4] for the serology and molecular biology of the Cromer blood group system. The purpose of the current review is to describe the role played by the DAF protein (containing the Cromer blood group antigens) in the physiological and pathophysiological functioning of the human body.

Complement System

Activation of complement

DAF functions in the complement system. This system is composed of more than 30 serum and cell-surface proteins, and it forms a major part of the innate immune system that protects the individual from pathogens and other foreign particles, helps to clear immune complexes and damaged cells from the body, and serves as a bridge to the adaptive immune system.[19,20] The complement cascade is a sequential series of enzymatic steps that is activated by one of three main pathways: the classical pathway begins with

antibody-antigen complexes, which bind to and activate C1; the alternative pathway has a constant low level "tick-over" activation that then amplifies on foreign surfaces; and the lectin pathway begins with mannan-binding lectin recognition of pathogens. All three pathways generate an enzyme complex called C3 convertase, which consists of C4b2a in the classical and lectin pathways and C3bBb in the alternative pathway. The C3 convertase cleaves the critical central component of the complement cascade, C3, into the fragments C3b and C3a. C3b itself forms part of the alternative pathway C3 convertase, C3bBb; hence this can also act as an amplification loop once complement is activated by any pathway. The downstream part of the complement cascade is a nonenzymatic construction of the terminal complement components C5b, C6, C7, C8, and C9 into the membrane attack complex C5b-9. The membrane attack complex inserts into cell membranes (of pathogens, or, if misdirected, of autologous RBCs) and creates a pore, allowing passage of water and ions and causing osmotic lysis of the RBC. In addition to this dramatic end result of killing cells through the membrane attack complex to protect the individual from pathogens and other particles, the complement system can protect the individual by several other effector mechanisms. First, two key soluble fragments of complement, C3a and C5a, are generated from the complement cascade, and these anaphylatoxins are potent proinflammatory molecules that are chemotactic and activate leukocytes. Second, the C3b itself can be covalently bound to pathogens or immune complexes, a process of opsonization that marks them for clearance by phagocytic cells, such as macrophages, that have receptors for C3b or its degradation fragments. Finally, complement forms a connection between the innate and adaptive immune responses, as the presence of C3d (a degradation product of C3b) on antigen substantially lowers the activation threshold for B cells through coligation of the B cell receptor along with a coreceptor complex composed of the C3d receptor (CD21) and the signaling protein CD19.[21]

Inhibitors of complement activation

Because of these destructive immune effector pathways of complement, and because there is continuous low-level generation of C3b through the "tick-over" mechanism (which uses C3 bound to water instead of C3b as part of the initial C3 convertase to start the process), the complement system is always poised to function against pathogens, but this leaves self tissues at substantial risk. The safe functioning of the complement system thus requires a robust system of inhibitors to prevent it from damaging self tissues. The major inhibitor targets are the C3 convertase enzyme and the membrane attack complex. The C3 convertase enzyme can be blocked by dissociation of its two components, C4b and C2a for the classical pathway and C3b and Bb for the alternative pathway; the C3 convertase decays spontaneously over time, and inhibitory proteins that increase the decay rate are said to have decay-accelerating activity. In addition, the C3b or C4b component of the C3 convertase can be cleaved by the serum protease factor I, in a reaction that requires a cofactor and inactivates the C3 convertase; proteins that can serve as the cofactor are said to have factor I–dependent cofactor activity. Two serum proteins, factor H and C4-binding protein, have both decay-accelerating activity and cofactor activity, with factor H acting on the alternative pathway C3 convertase and C4-binding protein on the classical pathway C3 convertase. Three membrane proteins have regulatory activity for the C3 convertase: CR1 (CD35) is the C3b receptor and also has both decay-accelerating activity and factor I–dependent cofactor activity for the classical and alternative pathway C3 convertases; DAF has decay-accelerating activity for both the classical and alternative pathway C3 convertases; and membrane cofactor protein (MCP, CD46) has factor I–dependent cofactor activity for the classical and alternative pathway C3 convertases. CR1 is distributed mainly on peripheral blood cells, whereas DAF and MCP are widely distributed on peripheral blood cells (except there is no MCP on RBCs), endothelial cells, and epithelial cells.

There are structural and genomic links of these five inhibitors as well as a sixth protein, the C3d receptor (CD21), as all are composed of multiple copies of a 60–amino acid domain termed the short consensus repeat (SCR) or the complement control protein (CCP) module. The proteins vary in size from DAF with four SCR domains to CR1 with 30 SCR domains. The genes for all six of these proteins are located in a region on the long arm of human chromosome 1 termed the regulators of complement activation (RCA) gene cluster.[22] The existence of a gene family to control complement activation at the C3 convertase step highlights both the critical nature of that control point in complement action and the importance of protecting self tissues from complement attack. An

additional control point for complement is at the level of the membrane attack complex. CD59, a GPI-anchored protein with a wide tissue distribution, inhibits assembly of the membrane attack complex, thus protecting RBCs from lysis.[23]

Structure and Function of DAF

Based on multiple biochemical and molecular biological studies, DAF is a 70,000-Da glycoprotein composed of four SCR domains of approximately 60 amino acids each, followed by a 67–amino acid serine/threonine-rich region that is heavily *O*-glycosylated and then a carboxyl-terminal GPI anchor that attaches DAF to the cell membrane through direct insertion of the phosphatidylinositol moiety into the outer leaflet of the lipid bilayer.[24] The GPI anchor is attached during an early posttranslational modification in which the original 28 carboxyl-terminal amino acids of DAF protein are cleaved off and the preformed GPI anchor is covalently attached to a serine residue at position 319.[25] Recent solution of the DAF structure through X-ray diffraction[26] shows that the four SCR domains have a rodlike arrangement, and that the serine/threonine-rich domain forms a rigid stalk, holding the functional SCR domains 17.7 nm above the plasma membrane (which is approximately 5 nm thick; Fig. 1). The single *N*-linked oligosaccharide is positioned between SCR 1 and SCR 2, and the multiple (average 11) *O*-linked oligosaccharides coat the serine/threonine-rich stalk (*O*-glycans attach to serine or threonine side chains) and probably protect it from proteolysis.

This X-ray diffraction structure of DAF, coupled with structural data on factor B, suggests that the larger C3 convertase complex contacts DAF in the region of SCR 2 and SCR 3, although more precise modeling will require the X-ray diffraction solution of the complex of DAF with C3 convertase. Biochemical data suggest that DAF does not bind the individual components of the C3 convertase (such as C3b or Bb) but binds the C3bBb or C4b2a complex, leading to dissociation of the Bb or C2a component, respectively.[27] Because DAF has a low affinity for the isolated C3b or C4b, DAF then is released and becomes available to inhibit another C3 convertase (Fig. 2).

On the functional level, DAF was identified and purified by its ability to protect RBCs from complement-mediated hemolysis.[9,11] Purified DAF was also found to have the ability to reincorporate into RBC (and other cell) plasma membranes and protect the cells from complement-mediated hemolysis.[28] The explanation of this ability came with the recognition that DAF was attached to cell membranes through a GPI anchor.[13,14] This reincorporation provides a potential route for effective delivery of DAF as a

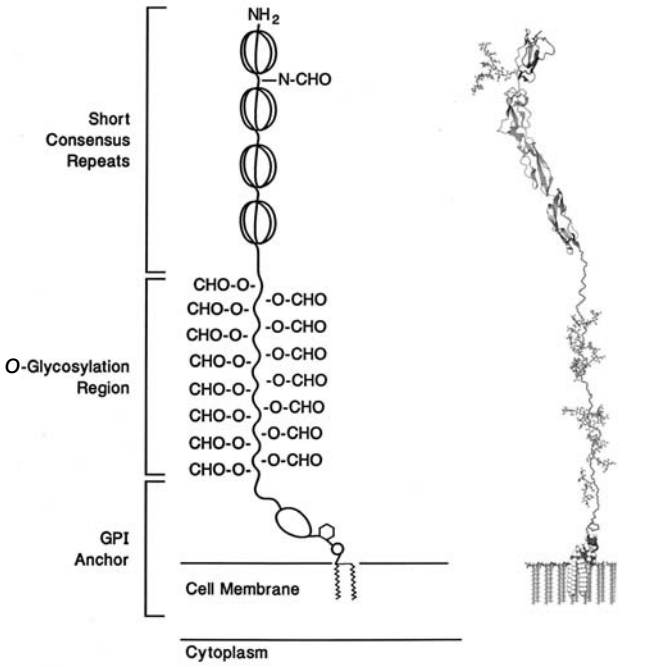

Fig. 1. Structure of the DAF protein carrying Cromer blood group antigens. The protein is shown in schematic form on the left, and a structural model based on atomic coordinates derived from X-ray diffraction data and other techniques is shown on the right (model reproduced from Lukacik, et al.[26] with permission from National Academy of Sciences, U.S.A., copyright 2004). The protein is composed of four SCR domains that carry out the complement regulatory function of DAF, a 67–amino acid serine/threonine-rich domain that is heavily *O*-glycosylated and forms a stalk projecting the DAF SCR domains above the cell surface, and a GPI anchor that inserts into the lipid bilayer of the plasma membrane.

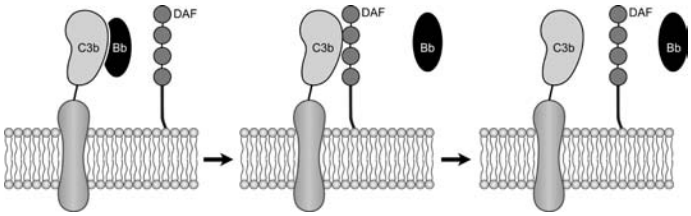

Fig. 2. Decay-accelerating activity of DAF for C3 convertase. **(Left panel)** C3b is shown covalently attached to a cell-surface protein, forming an alternative pathway C3 convertase by noncovalent association with Bb. **(Middle panel)** DAF binds to the C3bBb complex and dissociates Bb, destroying functional activity of the C3 convertase. **(Right panel)** DAF dissociates from the C3b, which makes it available to bind to and inhibit another C3 convertase. The same process occurs with the classical pathway C3 convertase, C4b2a, in which case DAF dissociates the C2a component.

therapeutic agent (discussed in a later section). One disease that highlights the function of DAF is paroxysmal nocturnal hemoglobinuria (PNH), an acquired hemolytic anemia in which affected peripheral RBCs have increased sensitivity to complement-mediated hemolysis, resulting in episodes of hemoglobinemia and hemoglobinuria. The cause of PNH is an acquired loss of GPI-anchored proteins in RBCs due to a mutation in the *X*-linked gene *PIG-A,* a gene that is required for the first step in the biosynthesis of the GPI anchor.[29] Because both DAF and CD59 are GPI-anchored proteins, both are reduced or absent on PNH cells and contribute to the increased sensitivity to complement, but only lack of CD59 leads to spontaneous intravascular hemolysis.[30]

Physiological Roles of DAF

Evidence from DAF knockout animals

The biochemical data on DAF demonstrated that it inhibits complement at the level of the C3 convertase. This was established by in vitro studies of isolated cells, usually RBCs. But what effect does DAF have on normal physiological function in an individual, and how does it protect against disease? The best evidence to answer these questions comes from studies comparing individuals with differing levels of DAF, with the most direct evidence from humans with the rare Inab phenotype (DAF-negative) and from DAF knockout mice.[31,32] In both the humans and the mice that lack DAF, the individuals are phenotypically normal. Neither humans nor mice lacking DAF show increased spontaneous deposition of C3 onto RBCs. Two important caveats must be considered in interpreting this finding (and not concluding that DAF is irrelevant). First, clearly, there are multiple proteins that control complement activation, as seen in the RCA gene family. Thus, lack of one protein could be compensated by other proteins, some of which could be up-regulated. Second, the set of RCA proteins differs in mice in several ways: DAF is expressed from two genes in the mouse, one of which encodes a GPI-anchored DAF protein that has a broad tissue distribution; and the other encodes a transmembrane-anchored DAF that is expressed predominantly in the testis.[33] The two groups that produced DAF knockout mice both targeted the GPI-anchored DAF as the version that parallels human DAF in its structure and expression pattern.[31,32] In addition, mice, but not humans, express a membrane protein termed Crry that has both decay-accelerating activity and factor I–dependent cofactor activity and is broadly expressed in tissues. Thus, the Crry expressed on mouse RBCs could substitute for DAF in protecting the cell from complement deposition; in humans, RBCs express CR1, which also has these two inhibitory functions for C3 convertase. Indeed, in vitro testing of the RBCs from Inab phenotype individuals, or from GPI-DAF knockout mice, using activators of the classical or alternative pathways, demonstrates that these DAF-deficient RBCs have increased C3b deposition although they do not show increased hemolysis (presumably protected from hemolysis by CD59).

Role of DAF in autoimmunity and inflammation

Because DAF protects cells from complement attack, lack of DAF could result in damage to self tissues, and hence be a factor in autoimmune and inflammatory diseases. However, as already mentioned, the Inab phenotype individuals have generally normal health. Nonetheless, one finding that has generated interest is that four of the seven (one probable) identified Inab individuals have a gastrointestinal disorder, including Crohn's disease, a protein-losing enteropathy, and a capillary angioma of the small intestine.[34] DAF is present on intestinal epithelium, so it is possible that this reflects an anti-inflammatory role of DAF, or else it could be an unrelated observation. The small number of Inab phenotype individuals makes it impossible to draw firm conclusions.

The recent production of DAF knockout mice by two groups,[31,32] as described earlier, has allowed a more extensive probing of the role of DAF in autoimmune and inflammatory disease and produced data that support a critical role for DAF in protection of self tissues. In an experimental model of inflammatory bowel disease based on dextran sulfate sodium–induced colitis, the DAF knockout mice showed markedly worse effects both clinically (bloody diarrhea, weight loss, and shortened colons) and histologically (increased neutrophil and mononuclear cell infiltration, greater epithelial cell destruction, and increased ulcerations) compared to the wild-type mice.[35] Two studies using experimental induction of immune complex-mediated glomerulonephritis demonstrated that the DAF knockout mice experienced more severe proteinuria, had more C3b deposition in glomeruli and more diseased glomeruli, and had more severe podocyte damage compared with wild-type control mice.[36,37] In a model of systemic lupus erythematosus (MRL/lpr mice) crossed into the DAF

knockout mice, researchers demonstrated that the DAF knockout group had greater lymphadenopathy and splenomegaly, higher levels of anti-chromatin antibodies, and more severe dermatitis with increased C3 deposition.[38] Finally, a recent study of DAF knockout mice in a model of experimental autoimmune myasthenia gravis showed that the DAF knockout mice had markedly reduced muscle strength along with increased C3b deposition and increased damage at the postsynaptic neuromuscular junction.[39] Thus, evidence from multiple disease models suggests that DAF plays an important role in protection against tissue damage in autoimmune diseases.

Role of DAF in pregnancy

Pregnancy is maintained despite an HLA incompatibility between mother and fetus. DAF is expressed on the placental trophoblast epithelial surface, where it could play a role in protecting the fetus from complement-mediated attack.[40] Interestingly, a Crry knockout mouse dies in utero with increased C3b deposition and placental inflammation, demonstrating the critical role that complement protection plays in pregnancy.[41] However, the *Crry* gene, which carries out functions of both DAF and MCP, is not present in humans. Both the Inab phenotype and the DAF knockout mice are viable through pregnancy, so DAF is not required for this purpose. But there is an unusual consequence of DAF expression on the placenta. In women who are immunized to Cromer antigens, maternal plasma antibody levels decrease or disappear during pregnancy[42] and the antibody can be recovered from the placenta,[43] but not from fetal RBCs; hence explaining the lack of HDN in Cromer antibody cases.

Additional roles of DAF

A number of other roles for DAF, some of which go beyond a function in the complement system, have been described. Several studies have shown that DAF can have an antiadhesive role, blocking adhesion of leukocytes to endothelial or epithelial surfaces[44,45] and thus modulating the inflammatory process. Cross-linking of DAF by antibody results in signal transduction in lymphocytes,[46,47] and this supports the possibility that ligands could initiate cell signals through DAF. DAF has also been identified as a ligand for CD97, a member of the seven-span transmembrane receptor family that is rapidly up-regulated upon leukocyte activation.[48] Furthermore, both DAF and CD97 were markedly up-regulated in brain tissue in multiple sclerosis lesions, suggesting that they might be involved in the inflammatory process in some way.[49] However, the physiological consequences of the DAF-CD97 interaction have not been elucidated.

DAF as a Therapeutic Agent

Given the in vivo properties of DAF in blocking the complement cascade, one can propose the use of DAF as an anti-inflammatory pharmaceutical agent. The key hurdle is to deliver DAF to the target. The most direct delivery method is to use DAF purified from a recombinant cell line (similar to production of recombinant factor VIII for hemophilia) and then administer it by a parenteral route, such as intravenous infusion. Alternatively, one could administer the recombinant DAF locally, such as by injection into an inflamed site. This has been tested in a guinea pig model of immune complex–mediated inflammation, in which intradermal injection of soluble DAF into the skin led to a marked reduction in the size of the inflammatory skin lesions.[50] Two novel methods of delivery of DAF have been described. DAF is attached to the cell through a GPI anchor, and under some circumstances DAF (and other GPI-anchored proteins) can transfer between cells, perhaps through direct cell-to-cell contact or through a plasma protein intermediate. One group used this property to transfer human DAF from RBCs to pig vascular endothelium.[51] In another interesting approach, a fusion construct was made between human DAF and a single-chain antibody fragment that targeted a mouse RBC antigen, and this construct successfully targeted DAF to mouse RBCs and protected them from hemolysis by human complement.[52]

A major focus of research on the therapeutic use of DAF has been in the area of xenotransplantation.[53] There is a shortage of human organs for transplantation, and this has encouraged the exploration of xenotransplantation as an option; pig organs are the most likely source for xenotransplantation due to anatomical and physiological similarities to human organs. However, a major barrier to pig-to-human transplantation is the hyperacute rejection that destroys the transplanted organ within minutes to hours; this is a complement-mediated process that is activated by naturally occurring antibodies in human and primate serum that react with pig endothelium, rapidly causing complement deposition and destruction of the vascular endothelium. These antibodies recognize a galactose-α1,3-galactose epitope (anti-Gal

antibodies) on pig vascular endothelium that is absent in humans and primates.[53] In an effort to block hyperacute rejection, several groups have generated DAF transgenic pigs and demonstrated that hyperacute xenograft rejection can be blocked in pig-to-primate heart, kidney, and liver transplants through use of organs from DAF transgenic pigs.[54–57] Although this is a major achievement, substantial additional immunological barriers remain, and these xenotransplants are destroyed by acute humoral rejection, a process that depends on the anti-Gal antibodies but does not appear to be primarily dependent on complement. Continuing research is addressing this problem through additional immunosuppressive regimens and the use of α1,3-galactosyltransferase knockout pigs that do not express the galactose-α1,3-galactose epitope.[53,58,59]

DAF as a Pathogen Receptor

Perhaps because it is broadly expressed on most cells and tissues, several viruses and bacteria have adapted DAF as a receptor for cell adhesion, invasion, or both. The major groups are enteroviruses and some pathogenic *Escherichia coli.* The genus of enteroviruses is in the family Picornaviridae (which includes poliovirus and hepatitis A virus, neither of which uses DAF as a receptor). The viruses in this family are small, nonenveloped, positive-strand RNA viruses, and they can cause aseptic meningitis, paralysis, myocarditis, and pneumonia. Some of the enteroviruses that bind DAF are echoviruses 7 and 12, enterovirus 70, coxsackievirus A21, and coxsackieviruses B1, B3, and B5. This was first demonstrated for several echoviruses[60,61] and was subsequently expanded to many additional enteroviruses.[62] DAF acts as a receptor to bind the virus to the cell, but it is not clear whether DAF by itself leads to infection or if an additional receptor is required. In some cases, it appears that the virus can be endocytosed into the cell through DAF and lipid rafts, which are microdomains of the membrane that are enriched in cholesterol, glycosphingolipids, and GPI-anchored proteins.[63] More generally, though, the data suggest that DAF functions to bind and concentrate the virus on the cell membrane, where it can be transferred to another receptor responsible for infection.[64,65] Thus, DAF serves to facilitate infection by this group of enteroviruses, and might directly lead to infection in some cases.

The other major group of pathogens that use DAF as a receptor are strains of *E. coli* that cause urinary tract and gastrointestinal disease. Pathogenic *E. coli* must adhere to and invade mucosal surfaces to produce infection. Many strains of *E. coli* contain an adhesin, located on bacterial fimbriae, that binds to DAF; this group of structurally related adhesins comprises the Dr family of adhesins.[66] The Dr adhesin name derives from the fact that the specific Dr adhesin binds to an epitope that is present in SCR 3 of DAF from individuals of the Dr(a+) Cromer phenotype but absent in the Dr(a–) phenotype.[67] Other members of the Dr adhesin family have different binding sites on DAF. The binding of the Dr adhesin–positive *E. coli* strains to DAF can result in internalization of the bacteria[68] and initiation of signaling pathways that can lead to cytoskeletal rearrangements[69] and cytokine production.[70,71]

Summary

The recognition that the Cromer blood group antigens reside on the DAF protein has been followed by an explosion of information about the serological, biochemical, and molecular biological basis of this blood group[4] and has provided molecular tools to probe the role of Cromer (DAF) in health and disease. DAF serves as a key regulator of the complement system, and thus plays a role in controlling autoimmune disease and inflammation. To protect self tissues from damage, DAF has a broad tissue distribution, and various bacteria and viruses have taken advantage of this by using DAF as a receptor. Exciting research is exploring the potential therapeutic use of recombinant DAF in inflammation and of DAF transgenic animals in xenotransplantation.

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Acute hemolytic transfusion reaction secondary to anti-Fy3

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A hemolytic transfusion reaction due to anti-Fy3 is reported in an African American patient with no history of sickle cell disease. This 82-year-old African American woman received two units of RBCs for anemia (Hb 7g/dL) on admission for a left hip fracture. On hospital Day 7, the patient underwent left hip endoprosthesis surgery; she received two units of RBCs on the second postoperative day due to Hb of 6.1g/dL. Her urine was dark during surgery and postoperatively. Her posttransfusion plasma was red. Her Hb dropped from 8.4 to 6.4 g/dL over 24 hours after the transfusion. Her total bilirubin rose to 4.0 mg/dL, with an LDH value of 1558 U/L and a haptoglobin of 10.9 mg/dL. Both the antibody detection test and the DAT were positive. An anti-Fy3 was identified in the serum and in the eluate. To the best of our knowledge, this is the first case of acute intravascular hemolysis due to anti-Fy3 in a patient without sickle cell disease. ***Immunohematology* 2005;21:48–52.**

Key Words: Duffy blood group system, intravascular hemolysis, transfusion reaction

The Duffy blood group system bears the name of a man with hemophilia who developed a novel antibody after multiple blood transfusions.[1] Two codominant alleles, *FYA* and *FYB,* are located on chromosome 1[2,3] and encode the antithetical antigens, Fy^a and Fy^b, distinguished only by a single mutated residue at position 42.[3–6] The corresponding antisera, anti-Fy^a and anti-Fy^b, define four phenotypes: Fy(a+b+), Fy(a+b–), Fy(a–b+), and Fy(a–b–). Additional high-prevalence Duffy antigens include Fy3 and Fy6. These antigens are recognized by monoclonal antibodies and have been mapped to specific locations in the Duffy glycopeptide. Thus, several anti-Fy3 monoclonal antibodies have been described; they recognize either a linear epitope on the third extracellular loop or a conformational epitope formed by several extracellular domains.[7,8] Conversely, anti-Fy6 monoclonal antibodies recognize an epitope in the N-terminal region, upstream of the Fy^a/Fy^b polymorphism.[9,10] Of note, the Fy6 antigenic determinant is defined by murine monoclonal antibodies only, and no human polyclonal antisera exist.[8,9] Fy(a–b–) individuals lack both Fy3 and Fy6 antigen expression on their RBCs. This Duffy-negative phenotype is commonly found in West Africans and African Americans.[11,12] Most of these individuals are genotypically *FYB* homozygotes carrying a point mutation in the GATA-1 motif, which specifically abolishes the transcriptional activity of Duffy genes in the erythroid cells.[13,14] However, the Duffy transcript can still be found in various extramedullary organs, expressed by the endothelial cells of postcapillary venules.[14] Therefore, it is thought that such individuals with low levels of Fy^b expression in nonerythroid cells would not develop Fy^b (or Fy3 or both) antibodies when challenged with Fy(b+) RBCs.

Anti-Fy3 is a rare RBC alloantibody first described by Albrey et al. in 1971.[15] This initial case was an Australian woman who, after receiving a blood transfusion during her second pregnancy, delivered a third baby with mild HDN. Investigation of her serum during prenatal testing of her third pregnancy led to the discovery of the antibody, and she was phenotyped as Fy(a–b–).[15] Subsequent reports have described the same antibody in a Cree Indian woman and in other Caucasian patients,[16–18] all of them with a history of transfusion or pregnancy. Interestingly, African American individuals whose RBCs phenotype as Fy(a–b–) usually develop anti-Fy3 after making anti-Fy^a or other alloantibodies.[19–22]

We report a case of an African American patient without sickle cell disease in whom solely anti-Fy3 was shown to cause a severe hemolytic transfusion reaction.

Case Report

An 82-year-old African American woman was admitted to the hospital for elective repair of a left femoral neck fracture. She had been in a motor vehicle accident approximately two months previously, at which time she sustained the injury and developed a traumatic left femoral deep venous thrombosis (DVT). She had been placed on Coumadin to treat the DVT. At the time of the current admission (Day 0) she had

Table 1. Laboratory values

Hospital day	Hb (g/dL)	RBCs transfused	LDH (U/L)	Bilirubin (mg/dL) Total	Bilirubin (mg/dL) Direct	DAT	Antibody detection test
0	7.5	2	–	0.6	0.1	Negative	Negative
1	9.3	–	–	–	–	–	–
5	10.7	–	–	–	–	–	–
6	9.6	–	–	–	–	–	Positive
7	9.1	–	–	–	–	–	–
9	6.1	2	–	–	–	–	–
	8.4	–	1558	4.0	0.4	Positive (C3d)	Anti-Fy3
10	6.4	–	–	–	–	–	Anti-Fy3
15	9.3	–	–	–	–	–	–
21	10.2	–	–	–	–	–	–

heme-positive stools and anemia with a Hb of 7.5 g/dL (baseline Hb 11 g/dL). (Table 1 summarizes the patient's laboratory data.) Her antibody detection test and DAT were negative. Her total bilirubin was 0.6 mg/dL. Her international normalized ratio (INR) was subtherapeutic at 1.2. The Coumadin was discontinued, and she received two units of RBCs on that day (Day 0). Her posttransfusion Hb rose appropriately to 10.7 g/dL by Day 5, when she underwent gastrointestinal endoscopy, which demonstrated only a duodenal arteriovenous malformation which was treated with heater probe.

On Day 7, the patient underwent endoprosthesis surgery. Her preoperative Hb was 9.1 g/dL. When a Foley catheter was inserted during surgery, grossly dark urine was noted in the Foley bag; this was attributed to traumatic catheter placement. Throughout the procedure, the patient had stable blood pressure and pulse readings, and her maximum temperature was 37.9°C. She had an estimated blood loss of 300 mL. That night, the patient sustained a fever of 38.2°C, but had no documented chills.

On Day 9 (postop day 2) the patient's Hb was 6.1 g/dL; a drop thought due to recurrent gastrointestinal bleeding. The patient was afebrile, and her vital signs were stable. She was transfused with two units of RBCs. One and one half hours after the transfusion of the second unit, red urine was noted in the Foley bag. Its analysis revealed free hemoglobin but no intact red cells. Her temperature rose from 36.3°C to 37.2°C. At this stage, hemolysis was suspected, and a workup showed increased LDH at 1558 (reference range 100–190 U/L), decreased haptoglobin at 10.9 mg/dL (reference range 16–200 mg/dL), and increased total bilirubin at 4 mg/dL (reference range 0.2–1.3 mg/dL) with a direct component of 0.4 mg/dL. The immunohematology workup is described below. The patient received intravenous saline to maintain urine output of approximately 200 mL/hr. The patient did well, with no evidence of renal dysfunction, and her subsequent hospital course was uncomplicated.

Materials and Methods

ABO and Rh typing as well as antibody detection and identification tests were performed by standard tube testing with LISS (Immucor, Inc., Norcross, GA) enhancement, using commercial reagents according to the manufacturer's protocol. The DAT was performed with polyspecific antihuman globulin and monospecific anti-IgG and anti-C3d reagents (Gamma Biologicals, Inc., Houston, TX). The patient's serum was tested against panels of commercial reagent RBCs (Gamma Biologicals, Inc.) to determine antibody specificities. LISS (Immucor, Inc.) and PEG (PeG™, Gamma Biologicals, Inc.) were used as potentiating media. Patient's serum and eluate prepared by an acid elution method (ELU-KIT II™, Gamma Biologicals, Inc.) were tested against panels of commercially available reagent RBCs (Gamma Biologicals, Inc., and Immucor, Inc.), and an inhouse frozen RBC inventory for antibody identification. The patient's serum was also tested against ficin-treated (Sigma, St. Louis, MO) RBCs, according to the AABB Technical Manual (14th ed.) protocol.

Results

The patient's antibody detection test was negative on admission (Day 0) and she was transfused with two units of crossmatch-compatible RBCs. On Day 9, she was again transfused with two units of crossmatch-compatible RBCs and, after transfusion of the second unit, a hemolytic transfusion reaction was reported, marked by red urine and a rise in temperature. The DAT was 1+ with polyspecific reagent, weakly positive with monospecific anti-C3d, and negative with anti-IgG. The posttransfusion reaction antibody detection test revealed a weak to 1+ reaction at the antiglobulin phase with LISS enhancement. The antibody detection test, performed on the pretransfusion sample, revealed a weak to 1+ reaction with both LISS and PEG

enhancements. Additional studies in LISS revealed positive reactions in 8 of 11 selected reagent RBCs at the antiglobulin phase. Ten of 11 reagent RBCs were positive, showing similar strengths of reactivity when the serum-to-cell ratio was increased. The only RBC that did not react was of the phenotype Fy(a–b–). This agglutination pattern was confirmed on two separate determinations with the same specimen. The eluate was reactive (weakly positive) in 8 of 9 panel RBCs. Once again, the only RBC that did not react was of the phenotype Fy(a–b–). All other antibodies were excluded using PEG enhancement. Serum tested against additional Fy(a–b–) selected RBCs showed no agglutination. When serum was tested against selected Duffy-positive RBCs treated with ficin, reactivity was seen in 9 of 9 RBCs at the antiglobulin phase. No agglutination was seen at that phase in 5 of 5 Fy(a–b–) ficin-treated RBCs. The presence of an anti-Fy3 was confirmed by the AABB Reference Laboratory at Gamma Biologicals (Houston, TX). The reference laboratory had excluded anti-Fy5 by testing the serum with Rh_{null} cells. The patient's pretransfusion RBC sample was phenotyped to be: D+, C–, E–, c+; M+, N+, S–, s+; P1+; Le(a–b+); K–; Fy(a–b–); and Jk(a+b–). The first unit of RBCs transfused upon admission was Fy(a–b–); the second was Fy(a–b+). Both units transfused postoperatively were Fy(a+b+), consistent with the ensuing hemolysis.

Discussion

Albrey et al.[15] first described anti-Fy3 after investigating a prenatal serum of a pregnant woman. Since that initial report, several other examples of anti-Fy3 have been described.[16–22] This is a clinically significant antibody associated with mild HDN in two offspring of the initially reported subjects.[15,16] Additional cases of this antibody developing after transfusion have been cited.[18,20] In some of those circumstances, it is not clear whether hemolytic complications occurred, although some of the subjects required blood transfusion. African American patients who developed anti-Fy3, with or without signs and symptoms of hemolysis, usually had concomitant alloantibodies within the Duffy system, especially anti-Fy^a.[19,20,23]

We are describing a patient who developed an intravascular hemolysis due to anti-Fy3, in the absence of any other alloantibody. This patient had a history of RBC transfusion. She was phenotyped during the current admission as Fy(a–b–). Fy(a–b–) is the principal phenotype in West Africans and African Americans and is characterized by the absence of Duffy antigens on RBCs. This is thought to represent an adaptive response to malarial infection, since *Plasmodium vivax* requires the presence of the Duffy antigen for RBC invasion.[11,24–26] In West Africa, *P. vivax* is conspicuously absent and 95 percent of individuals are Fy(a–b–). The molecular mechanism underlying this phenotype is thought to be the homozygosity for an *FYB* allele with a point mutation in the promoter region, which suppresses the expression of Duffy glycopeptide in RBCs.[13] However, Duffy antigens are produced in several other locations, including the endothelial cells of postcapillary venules and Purkinje cells of the cerebellum,[3,14,27] which would prevent the formation of anti-Fy^b and anti-Fy3 upon exposure to Fy(b+) RBCs.

In contrast, cases of the Fy(a–b–) phenotype described in Caucasians seem to be the result of a different mechanism, involving deletion, frameshift, or nonsense mutations in the open reading frame, while the GATA-1 binding site remains unmutated.[5,18] This results in an unstable mRNA with premature termination and lack of Duffy protein expression in all cells. The absence of this protein does not seem to have deleterious effects, even though it also functions as a nonspecific receptor for chemokines and therefore has been dubbed DARC (Duffy antigen receptor for chemokines).[11,28,29] DARC might act as an RBC surface scavenger to eliminate excessive chemokines produced under various pathologic circumstances[30,31] and is likely an important player in the pathogenesis of renal inflammation.[32–34] Patients with this type of Duffy gene silencing develop strong anti-Fy3.

In the case presented here, it is conceivable that a similar process could have induced the formation of anti-Fy3. While this is not the usual cause of the Fy(a–b–) phenotype in African Americans, there are several reports of the opposite phenomenon, in which a GATA-1 motif mutation was responsible for the Fy(a–b–) phenotype in Caucasian individuals.[18] We could not perform genotyping in this case to confirm this hypothesis. It was not possible to test the patient's anti-Fy3 with cord RBCs. Such testing would have been of interest because it is reported that anti-Fy3 made by people with the Caucasian Fy(a–b–) phenotype reacts strongly with cord RBCs, whereas that made by Blacks reacts weakly or does not react with cord RBCs.

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Novel molecular basis of an Inab phenotype

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The Cromer blood group system consists of ten high-prevalence and three low-prevalence antigens carried on decay-accelerating factor (DAF). DAF is found in the cell membranes of RBCs, granulocytes, platelets, and lymphocytes and is widely represented in other body tissues. Sequence analyses of DNA were performed on a blood sample from a 91-year-old Japanese woman whose serum contained an alloantibody to a high-prevalence antigen in the Cromer blood group system (anti-IFC). A blood sample from her daughter was also studied. Sequence analysis revealed a substitution of 508C>T in exon 4 of *DAF* in the proband. The proband's daughter was heterozygous for 508C/T. This study describes an Inab phenotype in which the 508C>T nonsense mutation is predicted to change arginine at amino acid residue 136 to a stop codon. This change is in SCR 3 of *DAF*. This study reports on the molecular basis of a new proband with the Inab phenotype who had no history of intestinal disorders. ***Immunohematology* 2005;21:53–55.**

Key Words: Cromer blood group system, decay-accelerating factor, DAF, CD55, Inab

The antigens of the Cromer blood group system are carried on decay-accelerating factor (DAF; CD55), which is a member of the regulators of complement activation family of proteins.[1] DAF has four homologous short consensus repeat (SCR) domains followed by an *O*-glycosylated serine- and threonine-rich region attached to a glycosylphosphatidylinositol (GPI) membrane anchor. DAF has a wide tissue distribution.[2]

The Cromer blood group system consists of ten high-prevalence and three low-prevalence antigens,[3] each of which, with the exception of IFC, is associated with a single amino acid change in *DAF*. RBCs of the Cromer null phenotype, also called Inab, totally lack DAF. Furthermore, RBCs of individuals with paroxysmal nocturnal hemoglobinuria (PNH) III lack all GPI-linked proteins and therefore lack DAF protein.[4]

All Inab phenotype propositi have been found because of the presence of anti-IFC that is directed at multiple epitopes on DAF. Only five Inab phenotype propositi have been described: three Japanese,[5-7] one Jewish American,[8] and one white American of Italian descent.[9] Three of these propositi had intestinal disorders, including protein-losing enteropathy,[5,7] Crohn's disease,[8] and blood capillary angioma of the small intestine.[7] The possibility of an association between DAF deficiency and intestinal disease is unlikely due to the absence of any such disorder in one of the Japanese propositi[6] and in an 86-year-old Inab phenotype woman and her 70-year-old brother.[9] A patient has been reported with an acquired and transient form of the Inab phenotype, in whom the DAF deficiency was limited to the RBCs and was associated with splenic infarcts.[10]

The molecular basis for the Inab phenotype has been determined for three Japanese probands. Two, including the original proband, Inab, had a nonsense mutation at nucleotide 216G>A in exon 2 that is predicted to encode Trp53Stop.[11] The third proband (HA) had a 263C>A substitution 24 bp upstream of the 3´ end of exon 2 that created a novel splice site giving rise to a 26-bp deletion in the mRNA transcript.[6]

This study describes the molecular basis of the Inab phenotype in another Japanese proband who had no signs or symptoms of any intestinal disorder.

Case Study

A 91-year-old Japanese woman was admitted to the hospital with a broken left hip. The patient had no history of an intestinal disorder. She had three pregnancies, and one transfusion. Her serum contained an antibody that reacted by the IAT with a high-prevalence antigen. The antibody was reactive with RBCs that were treated with papain, trypsin, or AET, but did not react with α-chymotrypsin–treated RBCs, PNH III RBCs, IFC– RBCs, and Dr(a–) RBCs. Two examples of RBCs with transiently-suppressed Cromer antigens were weakly reactive. The patient's serum also reacted with RBCs lacking other high-prevalence antigens, including those with the following phenotypes: AnWj–, At(a–), Ch–, Co:–3, Cs(a–), Di(b–), Emm–, En(a–), Er(a–), Fy:–3, Ge:–2,–3, Gy(a–), I–, In(b–), Jk:–3, JMH–, Jr(a–), Kn(a–), K_o, Lan–, LKE–, Lu:–3, LW(a–), McC(a–), PEL–,

PP1P^k–, Rg–, Rh_{null}, Sc:–1, U–, Vel–, Yk(a–), and Yt(a–). RBCs from the proband's daughter were reactive with the proband's serum.

The patient's RBCs are group A, D+, C+, E+, c+, e+; M+, N+, S–, s+; K–, Le(a+b–), Fy(a+b+), Jk(a–b+), Cr(a–), Tc(a–), Dr(a–), Es(a–), IFC–, WES(b–), UMC–, GUTI–, SERF–, ZENA–, and pedestrian in regard to the other high-prevalence antigens.

Materials and Methods

Samples

Peripheral blood samples were obtained from the proband and her daughter, following Institutional Review Board (IRB) approved protocols.

Sequencing of DNA

Genomic DNA was isolated from fresh whole blood (QIAamp Blood Kit, QIAGEN, Inc., Valencia, CA). The regions of *DAF* that include and flank exon 2, exon 3, exon 4, exon 5, and exon 6, and encode SCR 1 to SCR 4, were individually amplified using the sets of primers (Life Technologies Inc., Gaithersburg, MD) listed in Table 1. Two microliters of DNA per reaction were amplified using 5 U *Taq* DNA polymerase (HotStarTaq, QIAGEN, Inc.) in a 50-µL reaction mixture containing 1.5 mM MgCl2 (2.0 mM $MgCl_2$ for exon 2 primers), 1X PCR buffer, 0.2 mM dNTPs, and 100 ng of forward and reverse primers. Amplification was achieved over 35 cycles with a final extension time of 10 minutes. The PCR products were sequenced directly using an ABI 3100 sequencer (Applied Biosystems, Inc., Foster City, CA) and ABI Big Dye reagents with BD Half-Term (GenPak, Stony Brook, NY). The PCR products were sequenced in both directions.

The numbering of nucleotides and amino acids is according to Lublin et al.,[2,13] that is, nucleotide number 1 is the "A" of the initiation codon (AUG of *DAF*), whereas amino acid number 1 is the Asp that starts the mature protein.

Results

Sequence analysis of the PCR products showed a wild-type sequence for exons 2, 3, 5, and 6 and revealed a homozygous 508C>T mutation in exon 4 of *DAF* from the proband (Fig. 1). Sequence analyses of the proband's serologically incompatible daughter showed a heterozygous mutation (508C/T). This nonsense mutation is predicted to change Arg136 to a stop codon in the third SCR of DAF protein.

Table 1. Oligonucleotide primers used in the analysis of individual exons of DAF[12]

Name	Primer sequence (5´ ⇒ 3´)	Annealing temperature	Exon [expected size (bp)]
DAFEx2F	aacagcaaactgtgaggacac	60°C	exon 2 (294)
DAFEx2R	gatacattccattcccagaac		
DAFSCR2F	gggttattagggtccagataa	55°C	exon 3 (363)
DAFSCR2R	gagttctagcatgaatgaaggaaggg		
DAFX4F	tctaccacctcacatagttacc	60°C	exon 4 (266)
DAFX4R	cagtcatgaaactaacaatctcac		
DAFX5F	ggaaagtcaaatatgtgtgaatg	60°C	exon 5 (183)
DAFX5R	cagcctcacaatctgagtgc		
DAFSCR4F	gcatctcttgttggtaatgct	60°C	exon 6 (393)
DAFSCR4R	caacccacatatagacctgaggg		

Discussion

Like other people with the Inab phenotype, this proband was identified because of the presence of anti-IFC in her plasma. The mutation responsible for the Inab phenotype of this proband had not been

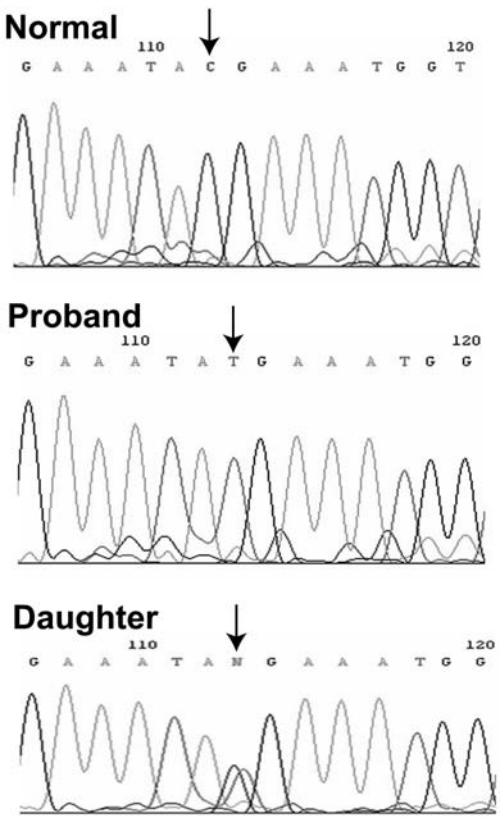

Fig. 1. Electropherogram of the relevant part of exon 4. A normal control (508C) is shown in the top panel, the proband (508T) in the middle panel, and the daughter (508C/T) in the bottom panel. (N = not interpreted by machine).

previously published. Molecular genotyping again shows that in the Cromer system, as in other blood group systems, different point mutations can be responsible for the null phenotype. It is interesting that there are three different silencing mutations that result in the Inab phenotype in the Japanese population when this phenotype is so rare.

Acknowledgments

We thank the staff of Canadian Blood Services: Ken Chin, Janet Csersko, Josephine Manansala, Sue Segura, and Esther Toews in Winnipeg, and Jacqueline Cote in Ottawa for technical assistance. We also thank Robert Ratner for preparing the manuscript and figures. The work was funded, in part, by NIH Specialized Center of Research (SCOR) grant in transfusion medicine and biology HL54459.

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Review: acute Donath-Landsteiner hemolytic anemia

A.F. EDER

The term paroxysmal cold hemoglobinuria (PCH) was first used in the early 1900s to describe immune hemolysis caused by the Donath-Landsteiner (DL) antibody, an IgG antibody with anti-P specificity that mediates biphasic hemolysis. The disease was aptly named for a recurrent complication in late-stage or congenital syphilis, observed as sudden attacks (paroxysms) of constitutional symptoms and hemoglobinuria precipitated by exposure to cold temperatures.[1(pp12–3)] Today, the diagnosis of chronic PCH in adults is extremely rare, its decline prompted by the advent of effective treatment for syphilis (Table 1). In contrast, the presence of DL antibodies should be immediately suspected in children with the abrupt onset of intravascular hemolysis that follows a recent viral or bacterial illness.[2–4] In children, the symptoms are not induced by cold exposure, resolve completely within a few weeks, and do not recur. Because the clinical presentation of acute disease in children differs from that of chronic PCH in adults, the condition is alternately referred to as Donath-Landsteiner hemolytic anemia (DL-HA).[3] Overall, immune hemolysis is an uncommon diagnosis in pediatrics, but DL antibodies are disproportionately implicated in 30 to 40 percent of cases of autoimmune hemolytic anemia in young children.[2–4] This review summarizes the clinical and serologic aspects of the acute transient form of hemolysis caused by DL antibodies in children.

Table 1. Classification of DL antibodies in a single institution[2]

Clinical classification	Age	Number (percent)
Acute	Children (< 13 years old)	30 (58%)
(Acute transient nonsyphilitic PCH)	Adult (> 27 years old)	14 (27%)
Chronic, nonsyphilitic PCH	Adult	3 (6%)
Chronic, syphilitic PCH	77 years old	1 (2%)
Unknown; incidental	67–82 years old	4 (7%)
		Total = 52

Pathogenesis

Julius Donath and Karl Landsteiner in 1904 were the first to attribute the temperature-dependent hemolysis in PCH to a cold-reacting autohemolysin and warm-reacting lytic factor.[1(pp12–3)] Their test for biphasic hemolysis after a cold-to-warm transition was the first immunohematologic test ever described and still serves as the definitive test for the Donath-Landsteiner autoantibody.[1(pp12–3)] The DL autoantibody is an IgG antibody that sensitizes RBCs at cold temperatures (< 20°C) by fixing the early components of complement, then dissociates from the RBCs at warmer temperatures. Complement activation is maximal at 37°C and proceeds to completion to produce hemolysis only after the warm incubation. Although initial incubation at low temperatures (0°C–20°C) is required for hemolysis in vitro, DL antibodies are also capable of sensitizing RBCs under physiologic conditions, even though peripheral body temperature rarely falls below 30°C. The mechanism by which DL antibodies produce such severe intravascular hemolysis is incompletely understood. Possible explanations include a heterogeneous population of DL antibodies with differential affinity in vivo and the ability of DL antibodies to initiate repeated cycles of complement activation by binding, dissociating, then reattaching to unaffected RBCs as blood circulates from cooler peripheral temperatures to warmer core temperatures.[2,3] In addition to the intrinsic characteristics of the DL antibody, patient-related factors, such as concurrent infection, may also contribute to an increased susceptibility to complement-mediated hemolysis in DL-HA.

The inciting stimulus for autoantibody formation is unknown, but infection or immune dysregulation may alter pathways that otherwise would suppress formation of antibodies against self-antigens.[5] Alternatively, pathogens may alter the RBC membrane to stimulate autoantibody formation or may possess antigens similar to those on RBC membranes so that

the resultant antibodies cross-react with RBCs (e.g., molecular mimicry).[6] DL-HA often occurs 1 to 3 weeks after a viral or bacterial infection and a wide variety of pathogens have been implicated in case reports (Table 2).[1(pp73–9)–4] More commonly, a specific etiologic agent is not identified, but an upper respiratory tract infection precedes the hemolysis in a vast majority of cases.[2,3] DL antibodies have specificity for the P antigen, a glycosphingolipid globoside that is also commonly expressed on microorganisms. The erythrocyte P antigen is the cellular receptor for parvovirus B19, and individuals who lack the P antigen (p or P^k phenotypes) are resistant to infection with the virus.[7,8] Acute infection with parvovirus B19 has been described in a child with DL-HA, but it does not appear to be a primary or underrecognized cause of DL-HA.[9,10] In a small case series of patients with DL antibodies, none had IgM antibodies against parvovirus B19, and IgG antibodies were not more common in the patients (7 of 13) compared to the control group (11 of 18).[10] Finally, several temporal clusters of acute DL-HA have been reported, but a common etiologic agent was not implicated in the cases.[2,4] Apparently, a wide variety of infections can trigger production of DL antibodies; moreover, increased awareness of the condition likely results in improved detection.[4] Rare examples of DL antibodies have been described in patients, typically adults, with other immunologic disorders, such as lymphoproliferative malignancies, collagen disease, myelodysplastic syndrome, delayed hemolytic transfusion reaction, and other types of autoimmune hemolytic anemia.[2,11–13]

Table 2. Pathogens implicated in case reports of acute DL-HA

Upper respiratory tract infection
Gastroenteritis, enteritis
Measles
Mumps
Chicken pox
Cytomegalovirus (CMV)
Epstein-Barr virus
Influenza virus
Adenovirus
Parvovirus B19
Coxsackie virus A9
Haemophilus influenza
Mycoplasma pneumoniae
Klebsiella pneumoniae
Measles vaccine

Clinical Presentation and Management

The typical presentation of DL-HA is a young child with a recent history of an upper respiratory tract infection or other acute illness associated with recurrent fever, and the passage of red-brown urine. Common characteristics in childhood cases from the two largest observational series describing DL antibodies are summarized in Table 3. DL-HA most often occurs in children under the age of 5 years (range, 8 months to 13 years).[2,3] In some reports, there is a slight male preponderance, with a male to female ratio of about 2:1.[2–4] Overall, DL-HA is an uncommon condition, but it may account for 30 to 40 percent of cases of autoimmune hemolytic anemia in young children.[2–4] The precise incidence and prevalence of DL-HA is unknown, and estimates based on small numbers of cases reported to blood services or reference laboratories, while informative, should be interpreted cautiously. Sokol et al. estimated the annual incidence of DL-HA as 0.4 per 100,000 for children under the age of 5 years.[2] The transient nature of the DL antibody, the level of awareness among primary care physicians, and the availability of the DL antibody are factors that potentially affect recognition and correct diagnosis of DL-HA.

The sudden onset of hemoglobinuria is reported in almost all cases of acute PCH, often accompanied by pallor, jaundice, and fever. The intravascular hemolysis and a rapidly progressing anemia may have a dramatic clinical presentation as high fever, shaking chills, and abdominal pain. Headache, nausea, vomiting, anorexia, and diarrhea may also occur. Physical findings included an enlarged liver and spleen in 25 percent of cases.[4] Typically, symptoms in children with acute DL-HA are not precipitated by cold exposure, in contrast to the presentation of chronic, syphilitic PCH in adults.

The degree of anemia is variable but may be severe in children with DL-HA. About one third of patients have hemoglobin concentrations of 5 g/dL or less (range: 2.5–12.5 g/dL) at presentation, which may decrease rapidly in the first 12 to 24 hours.[2–4] Differences in the severity of anemia among patients may reflect the characteristics of the DL antibody, such as serum titer or thermal range, or the interval of time between the onset of hemolysis and diagnosis. In addition, reticulocytopenia is often observed early in the course of the disease and the delayed hematopoietic response aggravates the initial anemia. Reticulocytopenia may result from viral suppression of the bone marrow or preferential destruction of reticulocytes by the DL autoantibody. However, the period of reticulocytopenia usually is brief and reticulocytosis commensurate with the degree of hemolysis promptly ensues. The peripheral blood

Table 3. Common findings in DL-HA in children

	Gottsche et al., 1990	Sokol et al., 1999
Number of cases (duration of study)	22 cases (4 years)	30 cases (37 years)
Patient age mean (range)	2.6 years (8 months–5 years)	3.5 (1–13 years)
Clinical presentation		
Percent (number) with precedent (< 3 weeks) infection	URI 77% (17/22) Other 23% (5/22)	URI 87% (26/30) Other 7% (2/30) None 7% (2/30)
Percent (number) with severe anemia (Hb < 5 g/dL)	27% (6/22)	40% (12/30)
Hemoglobin (g/dL)(range)	6.1 (4.4–8.8)	6.0 (3.4–12.9)
Percent (number) with hemoglobinuria	77% (17/22)	100% (30/30)
Serologic characteristics		
DAT		
Complement only	100% (22/22, at 37°C) 73% (16/22, at 20°C)	90% (27/30)
Complement + IgG	27% (6/22, at 20°C)	7% (2/30)
Not tested	–	3% (1/30)
Positive DL test		
Untreated RBC	59% (13/22)	93% (28/30)
Enzyme-RBC, only	41% (9/22)	3% (1/30)
Two-stage, only	–	3% (1/30)
Anti-P, if evaluated	NR	13/13 tested

URI = upper respiratory tract infection
NR = not reported

smear demonstrates RBC agglutination, polychromasia, nucleated RBCs, anisopoikilocytosis, occasional spherocytes, and erythrophagocytosis (Fig. 1). Erythrophagocytosis by neutrophils, rather than monocytes, is a relatively frequent phenomenon in PCH, but is rarely observed in other types of autoimmune hemolytic anemia.[14] The significance of this phenomenon to the RBC destruction that occurs is unclear, but extravascular hemolysis may contribute to the anemia and is further evidenced by occasional spherocytes on the peripheral blood smear. In addition, the monocyte monolayer assay (MMA) performed with mononuclear cells from patients with PCH demonstrated significantly more phagocytic activity than with those from normal individuals, suggesting enhanced reticuloendothelial cell function in the disease.[4] The practical significance of erythrophagocytosis, especially by neutrophils, in a young child is that it should trigger further investigation for DL-HA.[4] Additional serum chemistry findings in patients with DL-HA include increased LDH and unconjugated (indirect) bilirubin, decreased or absent haptoglobin, increased blood urea nitrogen (BUN), and increased serum creatinine.

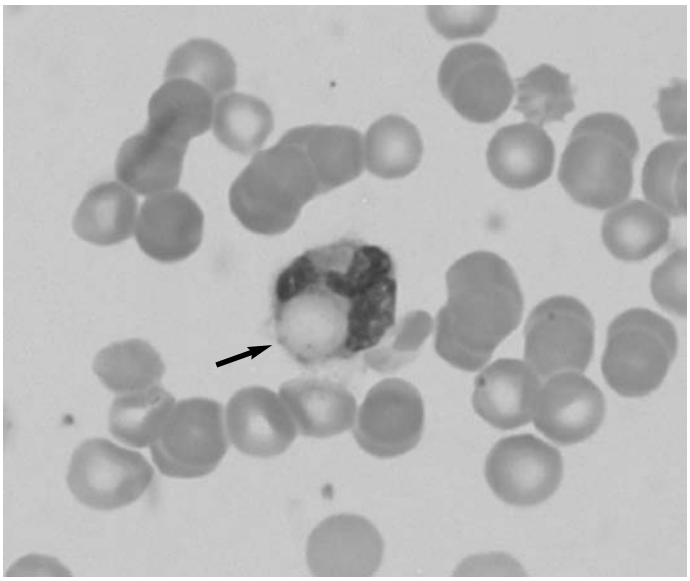

Fig. 1. Erythrophagocytosis in DL-HA. Peripheral blood from a 2-year-old girl with DL-HA and sudden onset of severe anemia (Hb 4.2 g/dL), showing erythrophagocytosis and RBC agglutination (100×; Wright stain).

Acute DL-HA usually resolves spontaneously and completely within several weeks and does not recur. There is a single case report of acute PCH occurring on two separate occasions in a child, each time after an upper respiratory tract infection, although the specificity of the antibody was not anti-P.[15] Treatment is supportive; glucocorticoids do not shorten the course of the disease. If glucocorticoids were started empirically for treatment of AIHA, they can be discontinued once the diagnosis of DL-HA is confirmed. Transfusion may be needed if anemia is severe.

DL antibodies do not interfere in routine pretransfusion and compatibility tests, because the causative autoantibody rarely causes RBC agglutination above 20°C. Patients with DL-HA typically demonstrate a DAT that is positive for complement, but negative for IgG, with a corresponding eluate that is negative. The antibody screen is usually negative, because DL antibodies do not react with RBCs under routine reaction conditions. DL antibodies may cause direct agglutination at 0°C or may be detected if the IAT is performed under strict conditions in the cold.

Although the specificity of the DL antibody is almost always anti-P, P– RBCs (i.e. p, P_1^k, or P_2^k) are extremely rare and are not available in routine practice for patients who require urgent transfusion. Fortunately, most patients with DL-HA who require

transfusion achieve a favorable clinical response and an adequate posttransfusion increment with P+ RBCs despite the presumed, or demonstrated, incompatibility. P– RBCs from rare donor registries have been used in case reports of PCH when hemolysis was severe and prolonged, although it is not clear whether transfusion of P– RBCs was beneficial or spontaneous recovery was coincident with transfusion.[16] Although cold-induced hemoglobinuria is rarely a feature of DL-HA, the patient should be kept warm during the transfusion and the use of a blood warmer is prudent despite the paucity of data that support this practice. Washing RBCs to remove residual complement may have theoretical benefit, but likely does not improve transfusion safety and often is not performed.[1(p396)] In rare cases of life-threatening anemia, plasmapheresis has been used to acutely remove IgG autoantibodies and alleviate symptoms.[17]

Donath-Landsteiner Antibodies

Donath-Landsteiner antibodies are typically IgG with anti-P specificity that demonstrate a low titer (< 32), a low thermal amplitude (< 20°C), and biphasic hemolysis. The vast majority of DL antibodies have these characteristics; however, the following exceptions have been described in case reports:

- *Specificity:* Biphasic IgG antibodies may demonstrate specificity for antigens other than P, such as anti-I, anti-p (anti-Gd), anti-i, and anti-"Pr-like."[1(p254)]
- *Thermal activity:* Biphasic IgG antibodies with anti-P specificity may demonstrate thermal activity above 20°C, agglutinate RBCs, or react by IAT at 37°C.[1(pp223–7),18–21]
- *Immunoglobulin subclass:* IgM antibodies may give a positive DL test, but these usually represent falsely positive results caused by monophasic hemolysis. Regardless, patients demonstrating IgM antibodies with biphasic hemolytic properties have been diagnosed as having PCH. The antibodies had anti-I, anti I^TP, or anti-P specificity.[1(pp195-6)]

Although there is no established consensus, diagnosis of DL-HA should require, at a minimum, a biphasic IgG antibody even if the specificity is not anti-P and evidence of intravascular hemolysis.[1(pp195–6)] The diagnosis of DL-HA should be questioned when the clinical history is atypical or the antibody is IgM or has other characteristics unusual for DL antibodies.

The IgG class of DL antibodies can be confirmed by performing the DAT under cold conditions, including cold washes to avoid eluting antibody. In addition, IgG may be demonstrated if IAT is performed in the cold with monospecific antihuman globulin reagents, although this test is susceptible to interference from normal cold agglutinins. In one report, a DL antibody was identified as IgG3, although it is not known whether this is a consistent finding in cases of DL-HA.[22] Finally, the MMA may be strongly positive in patients with PCH, suggesting the presence of IgG on the surface of their RBCs, and may be more sensitive than the IAT.[4,23]

Donath-Landsteiner Assay for Biphasic Hemolysis

The essential test for DL-HA is the Donath-Landsteiner assay for biphasic hemolysis, which can be performed either by the direct method using a sample of whole blood or by the indirect method using separated serum.[1(pp223–7),2] The patient's blood specimens must be maintained at 37°C after collection. Both assays first require an incubation of sample tubes in a melting ice bath (approximately 0°C) followed by an incubation at 37°C. The endpoint is the presence or absence of visible hemolysis in the plasma compared to control tubes kept at a constant temperature (0°C or 37°C). The direct test is an easy screening test but suffers from several limitations, as it requires more whole blood and is less sensitive than the indirect test.[4] Autologous RBC lysis may not be observed because of the protective effect of C3dg deposited on the surface of circulating RBCs during the hemolytic episode. Moreover, recent or ongoing hemolysis may deplete serum complement so that RBC lysis is not detected in the direct assay (Table 4).

The indirect test is performed with the patient's serum which has been maintained and promptly

Table 4. Limitations of DL tests

False-negative results
Direct DL test (lysis of autologous RBCs)
Low antibody titer
Low serum complement
Resistance to lysis due to C3dg
Indirect DL test (lysis of reagent RBCs)
Low antibody titer
Inhibition of DL antibody by globoside in normal serum
Autoadsorption of antibody during serum separation
False-positive results
Direct and indirect DL test
Monophasic lysis by an IgM autoantibody

separated at 37°C. A common method used for the DL indirect assay requires three sets of three tubes (Fig. 2).[24] The first set contains the patient's serum (row 1), the second set contains the patient's serum and fresh normal serum as an added source of complement (row 2), and the third set contains fresh normal serum as a control (row 3). Reagent ABO-compatible RBCs are added to all tubes. One tube from each set is incubated in a melting ice bath (approximately 0°C), then transferred to 37°C. The control tubes in each set are kept at a constant temperature, either 37°C or 0°C. Visible hemolysis in the patient's samples, with or without the additional complement, and the absence of hemolysis in all control tubes is a positive test result. Although maximal hemolysis is observed when the initial incubation is 0°C, DL antibodies in acute PCH may have thermal activity up to 24°C, but rarely cause lysis with higher temperatures in the initial incubation.

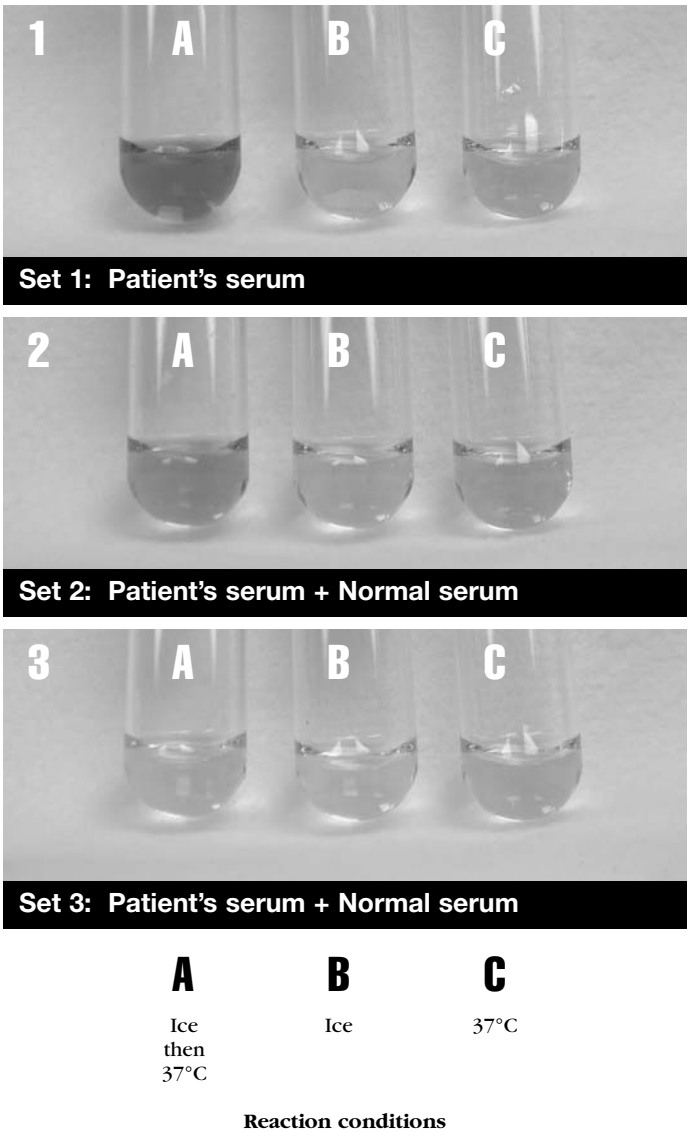

Fig. 2. Indirect Donath-Landsteiner test: sample conditions.

If RBCs with the p or P^k phenotype are available, they can be used as a negative control and should remain intact in the assay, confirming the expected anti-P specificity of the DL antibody. If an antiglobulin test is performed on the unlysed cells, antibody binding to P+ but not to p or P^k RBCs will be demonstrated. Because these RBCs are often not available, this additional testing may not be possible.

The most common cause of negative DL tests in suspected cases of DL-HA, with a classic clinical presentation and otherwise consistent laboratory findings, is the failure to detect the transient autoantibody that disappears quickly from the plasma during recovery from the acute illness. DL antibody titers quickly wane soon after recovery from the initial hemolytic episode. False-negative results may occur in both the direct and indirect test owing to low antibody titers. Because the indirect test evaluates the patient's serum only, false negatives may result from autoabsorption of antibody during serum separation or inhibition of antibody by neutralizing carbohydrate antigens (e.g., globoside) present in the added fresh serum (Table 4).

If the diagnosis of DL-HA is strongly suspected, but the indirect DL test is negative, the biphasic hemolysis test can be modified to increase its sensitivity by treating the reagent RBCs with enzymes, by performing a two-stage assay, or by testing for the DL antibody by the IAT. In addition, interpretation of the DL test may be difficult if the initial sample drawn for analysis is hemolyzed. In these cases, the extent of additional hemolysis during the assay may be gauged by the size of the red cell button after centrifugation. Alternatively, the two-stage test can be performed. In rare patients with classic DL-HA, the DL test was only positive with one of the following modified tests (Table 3)[2,3]:

- *Enzyme-treated RBCs:* The reagent RBCs may be treated with enzymes, such as 1% papain, which exposes more P antigen on the RBC membrane. Enzyme treatment, however, makes the RBCs more susceptible to lysis by cold agglutinins, and careful interpretation of appropriate controls is necessary.
- *Two-stage test:* For the two-stage test, the patient's serum is replaced with normal ABO-compatible serum after the first incubation at 0°C. Because antibody binding to the RBC occurs

only in the first phase, the patient's serum can be removed after this incubation occurs. Serum replacement not only removes the patient's serum that may have been hemolyzed at baseline, but also provides additional complement for the second phase of the assay at 37°C without diluting the sample.

- *IAT:* DL antibodies may be detectable by the IAT with antihuman globulin after the cold incubation with monospecific reagents.[1(p224)] However, interpretation of the test is problematic because the DL antibody or coincidental IgM antibody may cause direct agglutination at 0°C, and normal incomplete cold antibody may give false-positive results. A negative control with p or P^k phenotype RBCs should be used because of the propensity for false-positive results.

Conclusion

The diagnosis of DL-HA is readily made in most cases, but requires an index of clinical suspicion to initiate investigation, because the DL test is not performed routinely by most transfusion services. Despite the excellent reviews that have appeared in recent journals, the diagnosis is often still delayed, which confounds demonstration of the pathognomonic DL antibodies. A young child with a precedent viral illness and the sudden onset of intravascular hemolysis strongly suggests a diagnosis of DL-HA, even in light of a negative DL test. The most common cause of negative DL tests in acute cases is the failure to detect the transient autoantibody that disappears quickly from the plasma soon after the initial hemolytic episode. Drug-induced hemolysis (e.g., ceftriaxone), or possibly transfusion-associated hemolysis, may be associated with acute intravascular red cell destruction like acute DL-HA, but can be distinguished by the clinical history and immunohematologic findings.[2,25] On occasion, a cold-reactive IgM autoantibody may demonstrate apparent biphasic behavior; conversely, a DL antibody may appear to be monophasic because its thermal amplitude is close to physiologic temperatures. DL-HA is differentiated from cold agglutinin syndrome on clinical grounds and laboratory findings.[1(p196)] Although a rare disease, and rarely a diagnostic dilemma, DL-HA nonetheless requires prompt recognition for laboratory diagnosis and appropriate clinical management.

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Severe hemolytic anemia due to auto anti-N

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Auto anti-N is infrequently encountered and, in most reported cases, does not cause clinical hemolysis. This case reports an auto anti-N associated with severe hemolytic anemia (Hb = 2.7 g/dL) in a 6-year-old Caucasian girl with a history of vomiting, fever, and abdominal pain. Upon admission, she was found to have a metabolic acidosis, secondary to her severe anemia, with abnormal liver function tests. As in three other case reports, the autoimmune hemolytic anemia resolved, with disappearance of the auto anti-N, after corticosteroid therapy. ***Immunohematology* 2005,21:63–65.**

Key Words: hemolysis, autoantibody, anemia, antigens, MNS blood group system, autoimmune hemolytic anemia

Autoimmune hemolytic anemia can be caused by a variety of underlying illnesses. We report a case of an auto anti-N that caused a severe hemolytic anemia.

Case Report

A 6-year-old Caucasian girl (28.9 kg) with a 2-day history of vomiting, fever, and abdominal pain presented to her local physician and was found to be jaundiced. That evening, her emesis worsened and her urine was noted to be red. She presented to a local hospital with an initial Hct of 8% and abnormal liver function tests. She was transferred to our hospital; her admission laboratory values were Hb = 2.7 g/dL, Hct = 7.4%, WBC = 22.5 × 10^9/L, LDH = 7856 U/L (normal 470–900), AST = 587 U/L (normal 14–38), ALT = 434 U/L (normal 15–48), alkaline phosphatase = 232 U/L (normal 150–380), gamma glutamyl transferase (GGT) = 63 (normal 11–48), and total bilirubin = 12.3 mg/dL (Fig. 1). Peripheral smear showed lymphocytosis, with atypical lymphocytes, promyelocytes, myelocytes, and plasmacytoid lymphocytes; polychromasia; and varying shapes and sizes of RBCs with rouleaux. Anti-nuclear antibody (ANA) was positive (speckled pattern, 1:80). Tests for double-stranded DNA, Smith antigen (sm), ribonucleoprotein (RNP), Sjogren's syndrome A (SSA), and Sjogren's syndrome B (SSB) were all negative. The test for infectious mononucleosis was negative. EBV serologies were positive for IgG, but negative for IgM, suggestive of a previous, nonrecent infection. In addition, blood cultures were negative. At time of transfer, the patient was in severe metabolic acidosis, secondary to her severe anemia, with a pH of 7.17, pCO_2 of 23 mm Hg, pO_2 of 47 mm Hg, and bicarbonate (HCO_3) of 9.3 mmol/L.

Two units of group O, D– RBCs were released and transfused upon admission without pretransfusion testing. Antibody screening performed, after release of the units, showed a 1+ reaction with two cells of a three-cell screening panel, using the ID-MTS gel test (Ortho-Clinical Diagnostics, Raritan, NJ). Antibody panel results showed reactivity with N+ cells in saline room temperature and AHG tests using PEG (PeG™, Gamma Biologicals, Inc., Houston, TX). All other clinically significant antibodies were excluded. The autocontrol was positive in the same phases of testing as the panel cells. The DAT was 4+ with polyspecific AHG, 2+ with anti-IgG, and 3+ with anti-C3d. ABO/Rh typing was initially discrepant between forward and reverse typing, and the Rh control was positive. A 1-hour chloroquine diphosphate (Gamma-Quin,

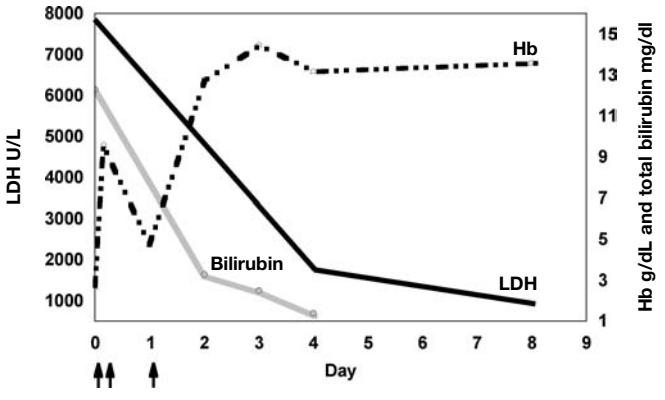

Fig. 1. Progression of patient's LDH, bilirubin, and Hb levels by day of hospitalization. Steroid therapy was initiated at the time of presentation to the hospital. The patient was transfused with two emergency-released uncrossmatched units of RBCs on Day 0 and one unit of crossmatched RBCs on Day 1. (Arrows indicate RBC transfusion.)

Table 1. Features of the cases of hemolytic anemia caused by auto anti-N

Study	Case no.	Age	Sex	Race	Initial Hb (g/dL)	LDH (U/L)	Bilirubin (mg/dL)	Underlying diagnosis	Steroids	Transfusion	Discharge Hb
Bowman et al.[1]	1	18	M	UNK*	6.9	2200	3.1	Infectious mononucleosis	Yes	Yes (2 units)	10.2 (24 days)
Dube et al.[2]	2	7	M	UNK*	6.0	UNK*	1.7	UNK*	Yes	Yes (1 unit)	10 (10 days)
Cohen et al.[3]	3	21	F	Caucasian	5.5	540	1.7	SLE†	Yes	No	12.8 (hospital stay unreported)
This report	4	6	F	Caucasian	2.7	7856	12.3	Questionable viral origin	Yes	Yes (3 units)	13.2 (4 days)

* Unknown
† Systemic lupus erythematosus

Gamma Biologicals, Inc., Houston, TX) treatment of the RBCs resolved the ABO typing and Rh control problem, and the patient's RBCs typed as group A, D+. The antibody was interpreted as a warm autoantibody with anti-N specificity. EDTA glycine-acid (EGA) treatment of the RBCs (Gamma EGA kit, Gamma Biologicals, Inc.) reduced the IgG reactivity from 2+ to w+, and the patient's EGA-treated RBCs typed 2+ for the N antigen. An AHG crossmatch of the two group O, D– emergency-released RBC units showed that one of the units was incompatible with the patient's serum. Further testing determined that the incompatible unit was N+; the other unit was N– and compatible. The patient received one additional unit of N–, AHG-crossmatch–compatible RBCs. Corticosteroid therapy was administered and the patient's Hb increased to 12.9 g/dL (Fig. 1).

Eight days after admission, a DAT of the patient's RBCs did not react with polyspecific AHG. The patient was discharged on corticosteroids with laboratory values of Hb = 13.2 g/dL, Hct = 36.7%, WBC = 9.2 × 10^9/L, LDH = 1773 U/L, and total bilirubin = 1.4 mg/dL. Follow-up transfusion testing two months later showed a negative antibody screen, negative DAT, and no ABO/Rh typing discrepancy.

Conclusion

After corticosteroid therapy, the autoimmune hemolytic anemia resolved and the auto anti-N disappeared. The etiology of the autoimmune hemolytic anemia was assumed to be related to the acute illness which was suspected to be of viral origin. There have been at least three other case reports of auto anti-N hemolytic anemia published (Table 1).[1-3] Two of the three reports were of pediatric patients (< 18 years old) and, as in our patient, all had antibody resolution after steroid therapy.[1,2]

Although infrequent, there have been at least 11 additional cases of naturally occurring auto anti-N in four healthy individuals and seven patients, all without evidence of clinical hemolysis or an associated etiology.[4-8] In addition, hemodialysis-associated anti-N–like antibodies have been described when formaldehyde was used to sterilize dialysis equipment. These antibodies were able to be adsorbed onto and eluted from NN RBCs as well as from formaldehyde-treated RBCs regardless of MN phenotype.[9-12]

Auto anti-N is infrequently encountered. In most reported cases, the antibody did not cause clinical hemolysis. In those few cases associated with hemolysis, as in the present case, the hemolysis resolved with steroid therapy.

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Analysis of SERF in Thai blood donors

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The Cromer blood group system consists of nine high-prevalence and three low-prevalence antigens carried on decay-accelerating factor (DAF). We recently described one of these Cromer high-prevalence antigens, SERF, the absence of which was found in a Thai woman. The lack of SERF antigen in this proband was associated with a substitution of nucleotide 647C>T in exon 5 of *DAF,* which is predicted to be a change of proline to leucine at amino acid position 182 in short consensus repeat (SCR) 3 of DAF. This study reports on PCR-RFLP analysis of the *SERF* allele with *Bst*NI restriction endonuclease on more than one thousand Thai blood donor samples. One new donor homozygous (647T) and 21 donors heterozygous (647C/T) for the *SERF* allele were found. Among this cohort of random Thai blood donors, the *SERF* allele frequency was 1.1 percent. Thus, like other alleles in the Cromer blood group system, *SERF* is found in a certain ethnic group. ***Immunohematology* 2005,21:66–69.**

Key Words: Cromer blood group system, decay-accelerating factor, DAF, CD55, SERF, high-prevalence antigen, Thais

The antigens of the Cromer blood group system are carried on decay-accelerating factor (DAF; CD55), which is a member of the regulators of complement activation family of proteins.[1] DAF has four homologous short consensus repeat (SCR) domains followed by an *O*-glycosylated serine- and threonine-rich region attached to a glycosylphosphatidylinositol (GPI) membrane anchor.[2] The Cromer blood group system consists of nine high-prevalence and three low-prevalence antigens.[3,4] Each antigen, with the exception of IFC, is associated with a single amino acid change in DAF. The location of the various Cromer antigens on DAF was accomplished by testing the corresponding antibodies against stable transfectants expressing full-length and deletion mutants of DAF.[5] $Tc^a/Tc^b/Tc^c$, Es^a, and WES^a/WES^b are located in SCR 1; Dr^a and SERF are within SCR 3; and Cr^a, UMC, and GUTI are within SCR 4.[4-8] The Dr(a–) phenotype is characterized by a single nucleotide substitution that is predicted to change serine to leucine at position 165 of DAF. This single nucleotide substitution exposes a cryptic splice site that leads to aberrant RNA splicing, causing a profound decrease in expression both of full-length RNA and of RBC-surface DAF, thus Cromer antigens are very weakly expressed on Dr(a–) RBCs.[9,10] RBCs of the Cromer null phenotype, known as Inab, totally lack DAF expression, with more than one mutation in the *DAF* gene reported.[9,11-13]

Interestingly, the absence of a high-prevalence Cromer blood group antigen is restricted to a certain ethnic group. The Cr(a–) and Tc(a–b+) phenotypes are found predominantly in the Black population.[1] Similarly, the Dr(a–) phenotype is restricted to Jews originating from the Bukharan area of Uzbekistan[9,14,15] and to the Japanese.[16-18] The mutation associated with the lack of the GUTI antigen has been found in 15 percent of Mapuche Indians in Chile.[8] We recently showed that the allele associated with an absence of the SERF antigen was present in 2 percent of Thai donors. The SERF polymorphism is associated with a single nucleotide change of 647C>T in exon 5, which is predicted to encode an amino acid change of proline 182 to leucine.[4]

This report describes a PCR-RFLP analysis for the *SERF* allele in 1041 Thai blood donors.

Materials and Methods

Samples

Three milliliters of peripheral blood samples were collected from each of 1041 healthy volunteer blood donors at the National Blood Centre, Thai Red Cross Society. Institutional Review Board (IRB) approval was obtained.

Genomic DNA extraction from blood

Genomic DNA was extracted from the buffy coat that was collected after centrifugation of the whole blood at 10,000 rpm for 5 minutes. RBC lysis buffer of 155 mM NH_4Cl, 10 mM $KHCO_3$, and 0.1 mM EDTA, pH 7.4, was added; the tube was centrifuged at 10,000 rpm for 10 minutes; and the supernatant was discarded. The white blood cell pellets were resuspended in 200 µL TE

buffer (25 mM Tris and 5 mM EDTA), and lysed by the addition of 10% SDS and proteinase K at 37°C for 1 to 2 hours. DNA was purified by chloroform-isoamyl alcohol extraction and precipitated by isopropanol at –20°C.[19] DNA was collected by centrifugation at 10,000 rpm at 4°C for 10 minutes, washed with cold 70% ethanol, and air-dried. DNA was solubilized in 50 µL of 0.5X TE buffer at 60°C for 10 minutes and stored at –20°C.

The quality and concentration of DNA was determined by comparison with known concentrations of λDNA/*Hin*dIII on a 0.7% agarose gel. Alternatively, the amount of DNA was measured by a spectrophotometer, using the absorbance at 260 nm, and calculated based on the definition that 1 OD_{260} of dsDNA is equivalent to 50 µg/mL DNA.

PCR-RFLP analysis with Bst*NI*

We used the PCR-RFLP assay previously described.[4] In brief, we used the sense primer, SERF 5´ (5´gtgtagtaaatattttaagataataacc 3´), that is located in intron 4, and the antisense primer, SERF 3´ (5´ cttacCTCTGCACTCTGGCACC 3´); the underlined C is a deliberate A>C change) that is located in exon 5 and extends 5 nucleotides into intron 5 (sequence data as per Douglas M. Lublin, MD, verbal communication). The primers were synthesized by Life Technologies, Inc. (Gaithersburg, MD). Two microliters of DNA (equivalent to 0.1 µg) per reaction were amplified using 5 U of *Taq* DNA polymerase (HotStarTaq, QIAGEN Inc., Valencia, CA) in a 50-µL reaction mixture containing 1.5 mM $MgCl_2$, 1X PCR buffer, 0.2 mM dNTPs, and 100 ng sense and antisense primers. PCR amplification was performed in a thermal cycler (9700, Perkin Elmer, Norwalk, CT) as follows: 35 cycles of 94°C for 20 seconds, 55°C for 20 seconds, and 72°C for 20 seconds, followed by a final extension of 10 minutes at 72°C. PCR products were analyzed on a 1.2% agarose gel and then subjected to digestion with *Bst*NI (New England Biolabs, Beverly, MA) overnight and analyzed on 8% polyacrylamide gel.

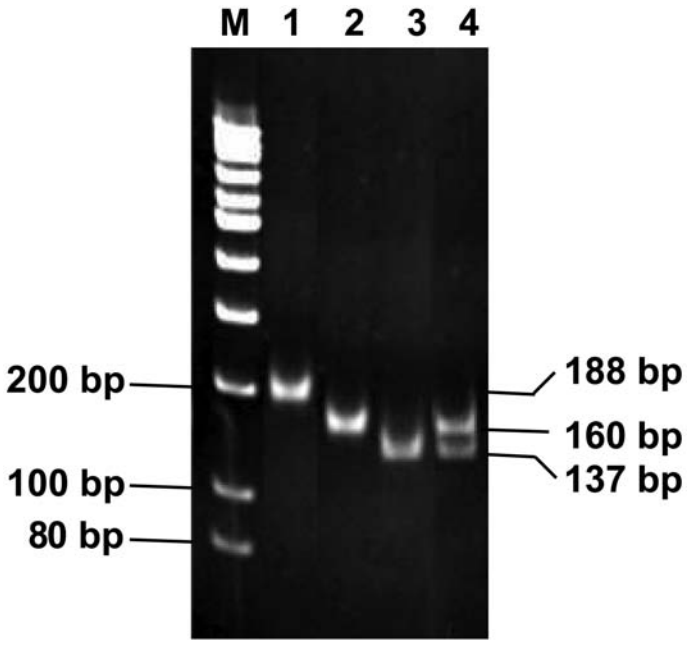

Fig. 1. PCR-RFLP analysis using *Bst*NI of DAF exon 5. **Lane M:** Molecular weight 100-bp DNA ladder marker. **Lane 1:** 188-bp uncut PCR amplicon. **Lane 2:** The digested PCR amplicon from a normal control DNA, which shows the 160-bp band. **Lane 3:** The digested PCR product of a sample, homozygous for the mutation, with a band of 137 bp. **Lane 4:** The digested PCR product from a sample, heterozygous for the mutation and wild-type with bands of 160 bp and 137 bp. The 28-bp band associated with wild-type and mutated alleles and the 23-bp band associated with the mutated allele ran off the gel and thus are not visible.

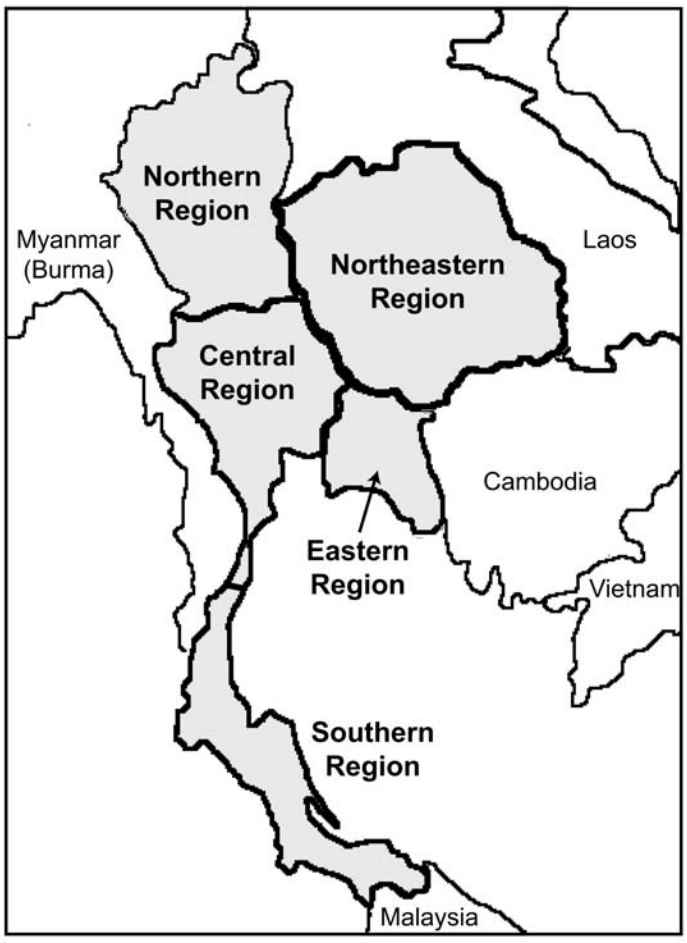

Fig. 2. Map of Thailand showing regions.

Results

The resulting PCR product of 188 bp, when digested with *Bst*NI, demonstrated one of three patterns (Fig. 1). Of the 1041 samples tested, which includes the 100 Thai donor samples previously reported,[4] we found one homozygous sample (in addition to the original proband) and 21 heterozygous samples. This represents an allele frequency of 1.1 percent in the 1041 random Thai donors. The allele was found in northern, eastern, and central regions, but not in the southern region of Thailand (Fig. 2). The region of origin was unknown for 35 of the donors. The *SERF*-negative allele was not found in 50 samples from the southern region. A summary of our findings is given in Table 1.

Table 1. Frequency of the *SERF*-negative allele in random Thai blood donors

Part of Thailand	Donors tested Number	(%)	Donors with *SERF*-negative allele Number	(%)
Northern	65	(6.2)	1	(0.8)
Northeastern	111	(10.7)	3	(1.4)
Eastern	19	(1.8)	1	(2.6)
Central	761	(73.1)	16*	(1.1)
Southern	50	(4.8)	0	(0)
No information	35	(3.4)	1	(1.4)
Total	1041		22	(1.1)

*One homozygous and 15 heterozygous samples

Discussion

SERF is a high-prevalence antigen in the Cromer blood group system and is associated with a proline residue at position 182 in *DAF*. The lack of SERF antigen on RBCs is associated with a single nucleotide mutation (647C>T) in exon 5, predicting an amino acid change (Pro182Leu) in the third SCR of *DAF*. Absence of the SERF antigen was found in the original Thai proband[4] and in one additional donor in this study. In the previous report,[4] two heterozygous samples were found in 100 random Thai donors. In this report, we tested 941 additional blood donors and confirmed that the mutated *SERF* allele has a frequency of 1.1 percent in Thailand. Thus, a total of 23 individuals (21 heterozygous random donors, one homozygous random donor, and the original proband) have been found to have the *SERF*-negative allele. Taken together, this represents an allele frequency of 1.2 percent (25/2084 × 100).

The SERF– phenotype joins other ethnically restricted Cromer phenotypes, the Cr(a–) and Tc(a–b+) phenotypes that are found predominantly in Blacks[1]; the Dr(a–) phenotype that is restricted to Jews originating from the Bukharan area of Uzbekistan[9,14,15] and to the Japanese[17,18]; and the mutation associated with the GUTI antigen, which has only been found in Mapuche Indians in Chile.[8] The Cromer phenotypes associated with certain ethnicities are summarized in Table 2. It should be noted that these ethnic associations have been determined by knowing the ethnicity of antibody producers and not by extensive testing in various ethnic groups.

Table 2. Antigens of the Cromer blood group system

Antigen name	Frequency	Predominant ethnic association(s)
Cr^a	High	Cr(a–) in Blacks 1 Spanish-American
Tc^a	High	Tc(a–) in Blacks Tc(a–) in Whites
Tc^b	Low	Tc(b+) in Blacks
Tc^c	Low	Tc(c+) in Whites
Dr^a	High	Dr(a–) in Uzbekistani Jews and Japanese
Es^a	High	No association
IFC	High	3 Japanese 2 Italian American 1 Jewish American
WES^a	Low	WES(a+) in Finns and Blacks
WES^b	High	WES(b–) in Finns and Blacks
UMC	High	Japanese
GUTI	High	GUTI– in Chileans
SERF	High	SERF– in Thais

Acknowledgments

We thank the staff of the National Blood Centre, Thai Red Cross Society, for providing the blood samples used in this study, and Robert Ratner for preparing the manuscript and figures. The work was funded in part by NIH Specialized Center of Research (SCOR) grant in transfusion medicine and biology HL54459 and the University Development Commissions (UDC) of Ministry of University Affairs, Thailand.

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Review: the Scianna blood group system

R.W. VELLIQUETTE

In 1962, an antibody to a high-prevalence antigen was described by Schmidt et al.[1] and named anti-Sm. The following year, Anderson et al.[2] described an antibody to a low-prevalence antigen and named it anti-Bu^a. These antigens were determined at that time to be independent of ABO, MNS, P, Lewis, Rh, Kell, Kidd, and Duffy blood group systems. In a study by Lewis et al.[3] in 1964, a hypothesis on the possible antithetical relationship between the antigens Sm and Bu^a was proposed. In 1967, Lewis et al.[4] presented further evidence supporting the hypothesis of this antithetical relationship and Sm and Bu^a were indeed determined to be products of allelic genes. Sm and Bu^a subsequently were shown to be genetically independent of other blood group loci and renamed Sc1 and Sc2, respectively.[5] The blood group system was named Scianna (SC), after the initial propositus. This conventional terminology shall be used henceforth in this review.

McCreary et al.[6] first described an antibody present in the serum of a patient from the Marshall Islands (Micronesia) with an apparent Sc:–1,–2 phenotype, which reacted with all RBCs tested except her own and those of her cousin with the same apparent Sc:–1,–2 phenotype. These findings suggested yet another antigen of high prevalence within the Scianna blood group system. These findings were substantiated when Nason et al.[7] discovered an antibody in the serum of a Caucasian man that reacted with all RBCs except his own and those of the two before-mentioned Sc:–1,–2 Micronesians. Adsorption and elution studies failed to reveal the presence of separable anti-Sc1 or anti-Sc2; therefore, the antibody was named anti-Sc3 and the corresponding antigen was named Sc3.

In 1967, Rausen et al.[8] described an antibody to a low-prevalence antigen present in five women who delivered children with HDN with detectable antibody on their cord RBCs. The antigen and corresponding antibody were named Rd and anti-Rd, respectively, after the Radin family in which they were first discovered. At that time, the Rd antigen was believed to be independent of any other blood group loci. In 1979, Lewis and Kaita[9] first described linkage of *Rd* and *RH* on chromosome 1 through linkage analysis and LOD scores. This newfound linkage of *Rd* and *RH* raised the possibility that the Rd antigen was part of the Scianna blood group system.[9] Spring demonstrated that anti-Rd gave a specific positive immunoblotting reaction to a broadly migrating component of approximate apparent molecular weight (M_r) 60 to 68 kD in Rd+ RBC membranes under nonreducing conditions that was not evident in Rd– RBC membranes.[10] This component was of a similar size to that defined by anti-Sc1 in the same membrane preparations.[10] Rd, however, remained autonomous and was assigned to the 700 series of low-incidence antigens by the ISBT.

After more than 20 years of researchers' suggesting the possibility that the Rd antigen belonged to the Scianna blood group system, Rd was finally proved to reside on the Scianna glycoprotein. This was achieved through the sequencing of the gene encoding the human erythrocyte membrane–associated protein (ERMAP) in 2002 by Wagner et al.[11] The Rd antigen was assigned to the Scianna blood group system and named Sc4.[12]

During the preparation of this review, an exciting discovery was made in the Scianna blood group system. A high-prevalence antigen named STAR was assigned into the blood group system and given the ISBT number 013005[12]; it became the fifth recognized antigen (Sc5) in the Scianna blood group system.

Properties of the Scianna Glycoprotein

Immunoblotting of RBC membranes under nonreducing conditions using anti-Sc1 and anti-Sc2 demonstrated that the Sc1 and Sc2 antigens are located on a RBC membrane glycoprotein with an approximate apparent M_r in SDS-PAGE between 60 and 68 kD.[13] The copy number of the Scianna antigens on RBCs has not been determined.

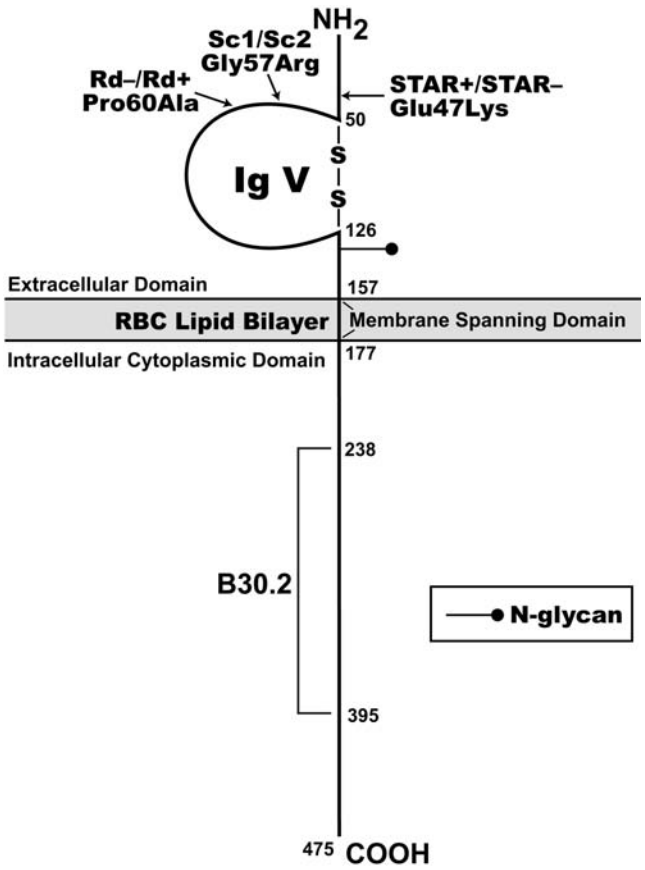


Fig. 1. ERMAP protein and Scianna polymorphisms. Figure is a modification of that found in *The Blood Group Antigen FactsBook.*[16]

Testing by hemagglutination has shown that both Sc1 and Sc2 are sensitive to treatment with pronase and require intact disulphide bonds for full antigen expression.[13] The Sc2-active glycoprotein is susceptible to endoglycosidase-F (Endo F) digestion, which suggests the glycoprotein carries one or more complex *N*-glycans that are necessary for the expression of the Sc2 antigen.[13] The Sc1-active glycoprotein appears to be resistant to Endo F digestion and does not require the presence of an *N*-glycan for expression. The Scianna glycoprotein does not have a substantial content of *O*-glycans and is not attached to the membrane by a glycosylphosphatidylinositol (GPI)-linkage.[13] Recently, given the historical data of the M_r of the Scianna glycoprotein and the chromosomal position of *SC,* a mutual compatibility was determined between the SC glycoprotein and ERMAP.[11] Thus, the antigens comprising the Scianna blood group system have been determined to be expressed by ERMAP.[11]

ERMAP is an erythroid single-pass adhesion/receptor transmembrane glycoprotein[11,14,15] (Fig. 1). The predicted protein is made up of 475 amino acids and is a member of both the IgV and B30.2 domain immunoglobulin superfamilies (IgSF).[14,15] Human ERMAP is homologous with murine ERMAP (73% identity or 346 of 475 amino acids and 14% conservative changes or 67 of 475 amino acids).[15] The extracellular domain contains one IgV fold that is homologous with the butyrophilin family of milk proteins, myelin/oligodendrocyte glycoprotein (MOG) autoantigen, and avian blood group antigens.[15] The intracellular region contains a conserved B30.2 domain that is encoded by a single large exon that is homologous with one encoding a similar domain in a diverse group of proteins including butyrophilin, pyrin, and MID1 (midline-1 protein).[15] ERMAP is highly expressed in erythroid cells in the fetal liver and adult bone marrow as well as in reticulocytes and circulating erythroblasts in fetal cord blood.[14] In one Northern blot, *ERMAP* mRNA was also weakly detectable in peripheral blood leukocytes, thymus, lymph nodes, and spleen, but was not detectable in most other adult tissues.[15] A possible explanation for this finding was variable erythroid cell contamination in the tissues harvested for the RNA extraction.[15] The exact physiological functions of ERMAP and the possible roles it may play in human disease pathogenesis still remain to be determined.

Scianna Antigens

The Scianna blood group system comprises five known, distinct antigens: Sc1 (Sm), Sc2 (Bu^a), Sc3, Sc4 (Rd), and Sc5 (STAR). Sc1 and Sc2 are an antithetical pair of high- and low-prevalence antigens, respectively. Sc3 and Sc5 are antigens of high prevalence. Sc4 (Rd) is an antigen of low prevalence with no known antithetical relationship to another Scianna antigen. ISBT terminology and the various phenotypes with prevalence of the Scianna system antigens are summarized in Tables 1 and 2,[16] respectively. The susceptibility and resistance of the antigens in the Scianna system to various enzymes and chemicals are listed in Table 3.[16,17]

Table 1. ISBT terminology for the Scianna blood group system

Antigen	ISBT name	ISBT number	Previous ISBT number
Sc1	SC1	013001	
Sc2	SC2	013002	
Sc3	SC3	013003	
Sc4 (Rd)	SC4	013004	700.015
Sc5 (STAR)	SC5	013005	

Table 2. Percentage prevalence of Scianna phenotypes

Phenotype	Caucasians	Blacks
Sc:1,–2	99%	100%
Sc:1,2	1%*	0%
Sc:–1,2	Very rare	0%
Sc:1,–2,4	Very rare	Very rare
Sc:1,2,4	Very rare	0%
Sc:1,–2,–5	Very rare	0%
Sc:–1,–2,–3 (Null)	Very rare	0%

*more common in Mennonites
Table 2 is a modification of that found in *The Blood Group Antigen FactsBook.*[16]

Addition of STAR to Scianna

Skradski et al.[18] first described an antibody found in a Caucasian man, R.S., with an apparent Sc:1,–2 phenotype, that reacted with approximately 200 donor RBC samples but not with his own, those from one brother (whose RBCs also typed Sc:1,–2) nor two examples of Sc:–1,–2 RBCs. Further findings were detailed by Devine et al.,[19] who described serologic evidence that Sc:–1,–2,–3 RBCs lack multiple high-prevalence antigens. The study by Devine et al. further described the before-mentioned patient with the Sc:1,–2 phenotype and two additional patients with the same Sc:1,–2 phenotype whose sera contained antibodies directed at a high-prevalence antigen. In each case, the antibody was reactive with all RBCs tested except the patient's own and Sc:–1,–2,–3 RBCs. The antibody present in each case was not mutually compatible. The antigen recognized by the antibody made by R.S. initially described by Skradski et al. in 1982 is now known as STAR.[20]

Scianna Alloantibodies and Their Clinical Importance

Alloantibodies directed at Scianna antigens are rare, and little is known about their clinical significance. However, most of what is known about them is derived from studies on Scianna autoantibodies and anti-Sc associated with pregnancy and HDN. Survival studies using radiolabeled RBCs have been published in only a few studies on the known Scianna antibodies.

One study suggested that anti-Sc3 may be clinically significant based on findings that used Cr^{51}-tagged Sc:1,–2 RBCs.[6] Similarly, one of the Scianna-related antibody cases (case #2) described by Devine et al.[19] was reported to have been implicated in a delayed hemolytic transfusion reaction, while another case (case #1)[19] demonstrated a good initial survival of Cr^{51}-labeled Sc3+ RBCs.

The first reported case of anti-Sc1 was described in a woman (Mrs. N.S.) who was also highly immunized to Rh D.[1] She was multiparous and all pregnancies after the first resulted in a fetus with HDN. However, in these cases it was impossible to assess the clinical role of anti-Sc1 in the HDN due to the presence of the anti-D in her serum.

Another example of anti-Sc1 was described by Kaye et al.[21] In this case report, anti-Sc1 associated with pregnancy was reported in a 28-year-old gravida-2 woman of Indian extraction. The anti-Sc1 was determined to be solely of the IgG3 subclass. The woman had no history of transfusion and no detectable atypical antibodies in her serum during her first pregnancy, and her RBCs typed Sc:–1,2. The woman delivered a full-term infant. At birth, the cord RBCs were positive (3+) in the DAT with anti-IgG (only). Anti-Sc1 was eluted from the cord RBCs. This antibody did not complicate her pregnancy and the newborn was not determined to be suffering from moderate or severe HDN. Neither the mother nor the infant required any transfusion and at 2 months of age the infant's RBCs were negative in the DAT.

The first case of HDN due to anti-Sc2 was reported by DeMarco et al.[22] The mother gave birth to a full-term

Table 3. Effect of enzymes and chemicals on intact RBC Scianna antigens

Antigen	Ficin/papain	Trypsin	α-chymotrypsin	Pronase	Sialidase	DTT 200 mM/50 mM
Sc1	Resistant	Resistant	Resistant	Weakened/sensitive*	Resistant	Sensitive/resistant
Sc2	Resistant	Resistant	Resistant	Sensitive	Resistant	Sensitive/resistant
Sc3	Resistant	Resistant	Resistant	Sensitive	Resistant	Sensitive/resistant
Sc4	Resistant	Variable	Variable	Sensitive	Resistant	Sensitive/resistant
Sc5	Presumed resistant	Presumed resistant	Presumed resistant	Presumed sensitive	Presumed resistant	Presumed sensitive/ resistant

*RBC membranes by immunoblotting
Sources for this information: *The Blood Group Antigens FactsBook*[16] and *Applied Blood Group Serology*[17]

infant. On Day 2 of life, the infant developed jaundice. The DAT on the stored cord RBCs revealed a weak positive reaction with anti-IgG. The DAT was performed again on a peripheral blood sample at Day 2 and it was 2+ with anti-IgG. An eluate prepared from the infant's RBCs contained anti-Sc2. The infant had a Hct of 45% on Day 2. Phototherapy was administered due to the jaundice and the infant was discharged after Day 3. Ten days later at a visit to the pediatrician, blood tests revealed that the infant's Hct had dropped to 22.6 %. The infant was monitored for the next 5 days. On Day 20, the Hct had dropped to 17.3 % without evidence of bleeding. The infant was hospitalized due to pallor, tachypnea, and tachycardia because of the severe immune hemolysis. The DAT on the infant's RBCs was 2+ with anti-IgG and anti-C3 reagents, and testing revealed an LDH level of 437 U/L (twice the upper limit of normal). The infant received 45 mL of crossmatch-compatible RBCs and the posttransfusion Hct increased to 27.8%. The infant was discharged the following day and remained stable without anemia or the need of transfusion.

As previously mentioned, Rausen et al.[8] reported on five cases of anti-Rd (Sc4). In that report, anti-Rd caused mild to moderate HDN with only one infant requiring exchange transfusion. The production of the anti-Rd in these cases seemed to be stimulated (only) by pregnancy. However, Lundsgaard et al.[23] reported on two additional cases of anti-Rd. The first case was an anti-Rd reported in a woman who was immunized by transfusion. This patient's RBCs were also positive in the DAT. A heat elution was performed on her RBCs, but the eluate reacted with all RBC samples tested regardless of their Rd phenotype. The second case reported was an anti-Rd found in a man who had never previously been hospitalized or transfused. The DAT on his RBCs was negative. In this case, it is believed that this anti-Rd was "naturally occurring." Before the addition of Rd to the Scianna blood group system, there were no cases of a "naturally occurring" Scianna alloantibody reported.

There are no reported data on the clinical importance of anti-STAR (Sc5). The characteristics and clinical significance of the Scianna alloantibodies are summarized in Table 4.[16,17]

Suppression of Sc Antigens and the Production of Sc Autoantibodies

In 1979, Tregellas et al.[24] described the first known auto anti-Sc1. The antibody-maker's RBCs were Sc:1,–2 with a weakly positive DAT due to bound IgG and C3d. The most interesting finding in this case was that the autoantibody produced was demonstrable in serum, but not in plasma. The antibody was IgG3 and, because it was detected in a healthy blood donor, it appeared not to be causing any in vivo RBC destruction. McDowell et al.[25] described two cases of autoimmune hemolytic anemia (AIHA) associated with auto anti-Sc1 due to transient suppression of Sc1. Similarly, Owen et al.[26] described a case of severe, acute AIHA in infancy found in a West Indian girl that was associated with auto anti-Sc1. The hemolysis was resistant to steroid therapy, but was resolved following splenectomy and two courses of high-dose IVIG given perioperatively. This case was novel because it was the first report of an auto anti-Sc1 that was detected by both serologic techniques and immunoblotting as well as having been the first instance of auto anti-Sc1 associated with severe, acute AIHA in childhood.

Peloquin et al.[27] described two cases of an apparent auto anti-Sc3 in two patients with suppressed Sc1 and Sc3 antigens. Both patients had an auto anti-Sc3–like antibody in both their serum and their plasma. The first case was a 64-year-old patient with lymphoma who had severe anemia. The patient's RBCs had a positive DAT due to weakly bound IgG and strongly bound C3d. The patient was transfused with five units of incompatible RBCs without any apparent adverse effect. The auto anti-Sc3 disappeared within 70 days of transfusion, but suppression of Sc1 and Sc3 antigens remained. The second case was a 54-year-old patient with Hodgkin's disease and congestive heart failure who had moderate anemia. The patient's RBCs had a positive DAT due to bound C3d only. The patient was transfused with six units of incompatible RBCs and the antibody

Table 4. Characteristics of Scianna alloantibodies

Antibody	Optimal technique	Complement binding	Immunoglobulin class	Transfusion reaction	HDN
Anti-Sc1	IAT	No	IgG	No	Positive DAT only
Anti-Sc2	IAT	No	IgG	No	Mild
Anti-Sc3	IAT	No	IgG	No	Mild
Anti-Sc4 (Rd)	IAT	No	IgG	No	Mild to severe
Anti-Sc5 (STAR)	IAT	Not known	IgG	None reported	None reported

Sources for this information: *The Blood Group Antigens FactsBook*[16] and *Applied Blood Group Serology*[17]

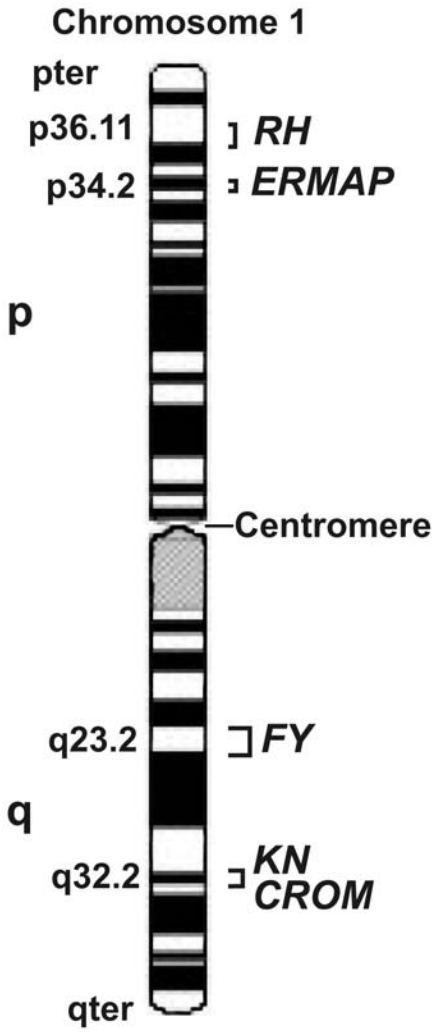


Fig. 2. Genomic location of *ERMAP* locus and its proximity to *RH* on chromosome 1. Figure is a modification of that found in *The Blood Group Antigen FactsBook.*[16] pter and qter denote the termini of the chromosome.

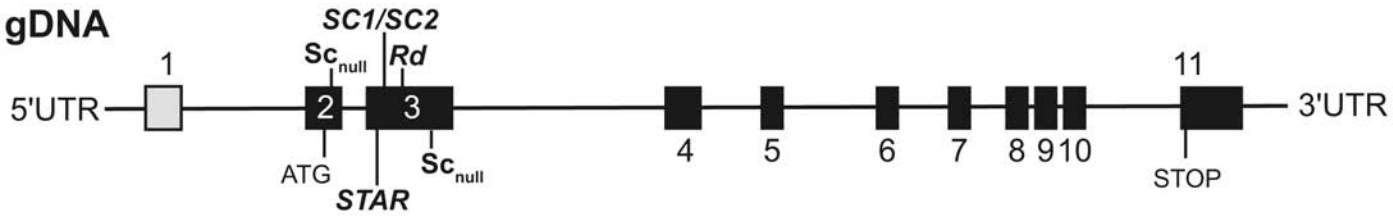
became slightly weaker. Follow-up studies were not possible in this latter case. These studies suggest that the formation of autoantibodies to Scianna antigens represents transient antigen suppression with concomitant antibody production.

Molecular Basis of Scianna

Over the span of years, Lewis et al.[9,28] showed through linkage data analysis that *SC* and *RH* were linked on chromosome 1p; however, they remained distinct. Noades et al.[29] provided independent confirmation of Lewis et al's claim of Rh linkage. The genomic location of the *SC* locus is 1p34[15,16] (Fig. 2). *ERMAP,* the gene encoding the Sc glycoprotein, is structured into 11 exons spanning 19 kbp of genomic DNA (gDNA).[11,15,16]

The genetic basis of the SC polymorphisms was determined by sequencing of *ERMAP*.[11,20] The polymorphisms underlying the Scianna antigens (except the Sc_{null} phenotype) are based on single nucleotide substitutions in *ERMAP* (Fig. 3).

At present, the reported molecular basis of the one Sc_{null} (Sc:–1,–2,–3) phenotype described resulted from a 2-bp deletion detected in exon 3 (307delGA). This induced frameshift caused a nonfunctional, truncated protein of 113 amino acids. Additionally, nucleotide substitutions 54C>T and 76C>T detected in exon 2 caused a His26Tyr amino acid substitution at codon 26.[11] There was no evidence for a second *ERMAP* allele, which suggested homozygosity for the *ERMAP* (54C>T, 76C>T, 307del2) allele.[11] However, this group suggested as an unlikely alternative that the *ERMAP* (54C>T, 76C>T, 307del2) allele could be *in trans* to a second,



Phenotype	Nucleotide Substitution
SC1/SC2	169 G>A in exon 3
Rd–/+	178C>G in exon 3
STAR+/–	139G>A in exon 3
Sc_{null}	54C>T 76C>T in exon 2, 307 delGA in exon 3

Fig. 3. *ERMAP* gene and the nucleotide substitutions underlying the Scianna polymorphisms and Sc_{null} phenotype. Figure is a modification of that found in *The Blood Group Antigen FactsBook.*[16]

nonfunctional allele that possessed gross structural changes that prevented amplification of exons 2 and 3.[11] The nucleic and amino acid sequence data obtained by Wagner et al.[11] were deposited in EMBL/GenBank/DDBJ under accession numbers AJ505027 to AJ505050.

Conclusion

The molecular basis of the Scianna antigens and the Sc glycoprotein was unknown until it was shown that they were carried on ERMAP, a glycoprotein of similar size. Sequencing of *ERMAP* allowed the elucidation of the molecular basis of the Scianna blood group system. The polymorphisms underlying the Scianna antigens are based on single nucleotide substitutions in *ERMAP,* with the exception of the Sc_{null} phenotype. The recent identification of the STAR antigen during the preparation of this review was only possible with this gene sequencing data. As STAR was a serologic curiosity buried in manuscript for more than 20 years, the molecular basis of Scianna may now allow for future elucidation of case studies that remain unresolved and also assist in discovery of the new.

Acknowledgments

The author is grateful to Marion E. Reid and Christine Lomas-Francis for their encouragement in the preparation of this manuscript and to Marion E. Reid for her critical review of the manuscript. The author also wishes to thank Robert Ratner for the preparation of the manuscript and figures.

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June 21–22 Florida Association of Blood Banks (FABB)

The 59th annual Florida Association of Blood Banks (FABB) conference will be held on June 21 and 22, 2005, at the Renaissance Vinoy Resort and Golf Club in St. Petersburg, Florida. The meeting's theme is Look Ahead Through Education. There will be two days of expert speakers, addressing topics such as medical and technical issues, donor recruitment, marketing, and networking. We are hoping to have a Fun Run along the bay for attendees. There will be many exhibitors with the latest in equipment, automation, and other blood bank necessities. For more information, visit the FABB Web site at http://www.floridaabb.com.

September 12–13 North Carolina Association of Blood Banks (NCABB)

The annual meeting of the North Carolina Association of Blood Banks (NCABB) will be held on September 12 and 13, 2005, in Winston-Salem, North Carolina. Registration forms are available at http://www.ncabb.org.

CLASSIFIED AD

Wake Forest University Baptist Medical Center in Winston-Salem, North Carolina, is accepting applications for an Assistant Manager position. Supervisor experience required. SBB preferred. Experience with FDA and AABB regulations desired. To apply, send a resume **directly to:** rjoseph@wfubmc.edu.

Manuscripts: The editorial staff of *Immunohematology* welcomes manuscripts pertaining to blood group serology and education for consideration for publication. We are especially interested in case reports, papers on platelet and white cell serology, scientific articles covering original investigations, and papers on new methods for use in the blood bank. **Deadlines** for receipt of manuscripts for consideration for the March, June, September, and December issues are the first weeks in November, February, May, and August, respectively. For instructions for scientific articles, case reports, and review articles, see "Instructions for Authors" in every issue of *Immunohematology* or on the Web. **Include fax and phone numbers and e-mail address with your manuscript.**

Notice to Readers: All articles published, including communications and book reviews, reflect the opinions of the authors and do not necessarily reflect the official policy of the American Red Cross.

Immunohematology

JOURNAL OF BLOOD GROUP SEROLOGY AND EDUCATION

Instructions for Authors

SCIENTIFIC ARTICLES, REVIEWS, AND CASE REPORTS

Before submitting a manuscript, consult current issues of *Immunohematology* for style. Type the manuscript on white bond paper (8.5" × 11") and double-space throughout. Number the pages consecutively in the upper right-hand corner, beginning with the title page. Each component of the manuscript must start on a new page in the following order:

1. Title page
2. Abstract
3. Text
4. Acknowledgments
5. References
6. Author information
7. Tables—see 7 under Preparation
8. Figures—see 8 under Preparation

Preparation of manuscripts

1. Title page
   - A. Full title of manuscript with only first letter of first word capitalized (bold title)
   - B. Initials and last name of each author (no degrees; all CAPS), e.g., M.T. JONES and J.H. BROWN
   - C. Running title of ≤ 40 characters, including spaces
   - D. 3 to 10 key words
2. Abstract
   - A. One paragraph, no longer than 300 words
   - B. Purpose, methods, findings, and conclusions of study
3. Key words—list under abstract
4. Text (serial pages)

   Most manuscripts can usually, but not necessarily, be divided into sections (as described below). Results of surveys and review papers are examples that may need individualized sections.
   - A. Introduction

     Purpose and rationale for study, including pertinent background references.
   - B. Case Report (if study calls for one)

     Clinical and/or hematologic data and background serology.
   - C. Materials and Methods

     Selection and number of subjects, samples, items, etc. studied and description of appropriate controls, procedures, methods, equipment, reagents, etc. Equipment and reagents should be identified in parentheses by model or lot and manufacturer's name, city, and state. Do not use patients' names or hospital numbers.
   - D. Results

     Presentation of concise and sequential results, referring to pertinent tables and/or figures, if applicable.
   - E. Discussion

     Implications and limitations of the study, links to other studies; if appropriate, link conclusions to purpose of study as stated in introduction.
5. Acknowledgments

   Acknowledge those who have made substantial contributions to the study, including secretarial assistance; list any grants.
6. References
   - A. In text, use superscript, arabic numbers.
   - B. Number references consecutively in the order they occur in the text.
   - C. Use inclusive pages of cited references, e.g., 1431–7.
   - D. Refer to current issues of *Immunohematology* for style.
7. Tables
   - A. Head each with a brief title, capitalize first letter of first word (e.g., Table 1. Results of ...), and use no punctuation at the end of the title.
   - B. Use short headings for each column needed and capitalize first letter of first word. Omit vertical lines.
   - C. Place explanations in footnotes (sequence: *, †, ‡, §, ¶, **, ††).
8. Figures
   - A. Figures can be submitted either by e-mail or as photographs (5″ × 7″ glossy).
   - B. Place caption for a figure on a separate page (e.g., Fig. 1. Results of ...), ending with a period. If figure is submitted as a glossy, place first author's name and figure number on back of each glossy submitted.
   - C. When plotting points on a figure, use the following symbols if possible: ○ ● △ ▲ □ ■.
9. Author information
   - A. List first name, middle initial, last name, highest academic degree, position held, institution and department, and **complete** address (including zip code) for **all** authors. List country when applicable.

SCIENTIFIC ARTICLES AND CASE REPORTS SUBMITTED AS LETTERS TO THE EDITOR

Preparation

1. Heading—To the Editor:
2. Under heading—title with first letter capitalized.
3. Text—write in letter format (paragraphs).
4. Author(s)—type flush right; for first author: name, degree, institution, address (including city, state, ZIP code, and country); for other authors: name, degree, institution, city, and state.
5. References—limited to ten.
6. One table and/or figure allowed.

Send all manuscripts by e-mail to:
Marge Manigly at mmanigly@usa.redcross.org

Becoming a Specialist in Blood Banking (SBB)

What is a certified Specialist in Blood Banking (SBB)?

- Someone with educational and work experience qualifications who successfully passes the American Society for Clinical Pathology (ASCP) board of registry (BOR) examination for the Specialist in Blood Banking.
- This person will have advanced knowledge, skills, and abilities in the field of transfusion medicine and blood banking.

Individuals who have an SBB certification serve in many areas of transfusion medicine:

- Serve as regulatory, technical, procedural, and research advisors
- Perform and direct administrative functions
- Develop, validate, implement, and perform laboratory procedures
- Analyze quality issues, preparing and implementing corrective actions to prevent and document issues
- Design and present educational programs
- Provide technical and scientific training in blood transfusion medicine
- Conduct research in transfusion medicine

Who are SBBs?

Supervisors of Transfusion Services	Managers of Blood Centers	LIS Coordinators Educators
Supervisors of Reference Laboratories	Research Scientists	Consumer Safety Officers
Quality Assurance Officers	Technical Representatives	Reference Lab Specialist

Why be an SBB?

Professional growth Job placement Job satisfaction Career advancement

How does one become an SBB?

- Attend a CAAHEP-accredited Specialist in Blood Bank Technology Program **OR**
- Sit for the examination based on criteria established by ASCP for education and experience

Fact #1: In recent years, the average SBB exam pass rate is only 38%.

Fact #2: In recent years, greater than 73% of people who graduate from CAAHEP-accredited programs pass the SBB exam.

Conclusion:

The **BEST** route for obtaining an SBB certification is to attend a CAAHEP-accredited Specialist in Blood Bank Technology Program

Contact the following programs for more information:

Program	Contact Name	Contact Information
Walter Reed Army Medical Center	William Turcan	202-782-6210; William.Turcan@NA.AMEDD.ARMY.MIL
Transfusion Medicine Center at Florida Blood Services	Marjorie Doty	727-568-5433 x 1514; mdoty@fbsblood.org
Univ. of Illinois at Chicago	Veronica Lewis	312-996-6721; veronica@uic.edu
Medical Center of Louisiana	Karen Kirkley	504-903-2466; kkirkl@lsuhsc.edu
NIH Clinical Center Department of Transfusion Medicine	Karen Byrne	301-496-8335; Kbyrne@mail.cc.nih.gov
Johns Hopkins Hospital	Christine Beritela	410-955-6580; cberite1@jhmi.edu
ARC-Central OH Region, OSU Medical Center	Joanne Kosanke	614-253-2740 x 2270; kosankej@usa.redcross.org
Hoxworth Blood Center/Univ. of Cincinnati Medical Center	Catherine Beiting	513-558-1275; Catherine.Beiting@uc.edu
Gulf Coast School of Blood Bank Technology	Clare Wong	713-791-6201; cwong@giveblood.org
Univ. of Texas SW Medical Center	Barbara Laird-Fryer	214-648-1785; Barbara.Fryer@UTSouthwestern.edu
Univ. of Texas Medical Branch at Galveston	Janet Vincent	409-772-4866; jvincent@utmb.edu
Univ. of Texas Health Science Center at San Antonio	Bonnie Fodermaier Linda Smith	SBB Program: 210-358-2807, bfodermaier@university-health-sys.com MS Program: 210-567-8869; smithla@uthscsa.edu
Blood Center of Southeastern Wisconsin	Lynne LeMense	414-937-6403; Irlemense@bcsew.edu

Additional information can be found by visiting the following Web sites: www.ascp.org, www.caahep.org, and www.aabb.org

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