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The complement system first came to the attention of scientists following work by Koch and Pasteur on transmissible diseases in the latter half of the 19th century. Leading microbiologists such as Paul Ehrlich, Jules Bordet, and George Nuttall were stimulated to learn more about the human body's mechanisms of protection against infectious disease. In 1888, Nuttall noted that sheep blood was mildly bactericidal for anthrax bacilli, but that this property disappeared rapidly if the blood was heated to 55°C or kept at room temperature. The heat-labile bactericidal activity was named “alexin.”

In 1894, other laboratories demonstrated that serum from guinea pigs that had recovered from cholera would protect normal guinea pigs from cholera if it was injected along with the live cholera bacteria. Importantly, it was shown by in vitro studies that the cholera bacteria were only killed by fresh immune serum, and not by heat-inactivated immune serum. However, it was noted that the heat-inactivated immune serum would protect normal guinea pigs from exposure to cholera bacteria. Bordet subsequently showed that the activity of the heat-inactivated serum could be restored in vitro by the addition of a small amount of fresh normal serum that by itself lacked bactericidal activity. These results indicated that the bactericidal activity depended on both a heat-stable sensitizing substance in immune serum and a heat-labile cytotoxic factor present in normal (as well as immune) serum. Bordet attributed the heat-labile activity to the alexin bactericidal factor. In 1899, Ehrlich put forward a humoral immunity scheme substituting the terms “complement” for alexin and “amboceptor” for the heat-stable immune sensitizer.

It soon became evident that complement consisted of more than two serum components. By the 1920s it was concluded that there were at least four serum fractions responsible for the complement activities of serum. To maintain order in this proliferation of complement components, each factor was assigned a number in the order in which it was discovered, i.e., C1, C2, C3, and C4. It later became clear that the factors reacted sequentially, but not in the order of their discovery. In joint international meetings it was decided to not change the numbers already assigned to newly defined complement factors, but rather to display their order of reactivity as this evolved from ongoing research. Thus by the late 1930s, the reactivity sequence for the first four complement factors was shown as: C1 → C4 → C2 → C3.

The complement story had been developed from the perspective of infectious disease, i.e., that it was part of the safety net that protected humans from the ravages of pathogenic microorganisms (bacteria, fungi, viruses). Thus it was postulated that the complement system was activated by the body’s response to attack by pathogenic microorganisms, presumably by production of immune reactants (antibodies) against the attacking microorganisms. The starting point of the complement response presupposed an infection-related activator. Because the protective response was hypothesized from the infectious disease perspective (earlier research had validated that hypothesis), the C1 → C4 → C2 → C3 sequence was dubbed the “classical pathway” of entry into the complement system.

However, it had been noticed that certain strains of bacteria and yeast could activate the complement system without having induced antibodies. In 1913, it was shown that cobra venom would mediate complement-like RBC lysis in the absence of immune antibodies. These studies suggested that the involved serum components that differed from those of the classical pathway. In fact, these findings presaged what
40 years later would be named the “alternative pathway” based on work by other researchers. They included Dr. Louis Pillemer, who described a previously unidentified serum protein named “properdin,” which was said to bind to bacteria and yeast, activating complement without requiring the presence of antibody. In 1957, his claim to have discovered a “properdin pathway” to complement activation was denounced by most contemporary complementologists on the grounds of serious technical inadequacies. Pillemer was deeply hurt by the scathing criticism. He became distraught over the damage done to his personal and professional reputation, and took his own life several months later. The tragedy deepened when subsequent work proved Pillemer’s original findings to have been correct. By 1967, properdin had been reinstated as an important factor in alternative pathway complement activation.

**Distinctive Differences Between the Classical and Alternative Pathways of Complement Activation**

As we have seen, the classical pathway depends on activation by antigen-specific antibodies reacting with the four principal complement factors (C1, C2, C3, and C4). Table 1 (adapted from Pangburn’s chapter on the alternative pathway) provides numerous examples of pathogens and particles of microbial origin, as well as nonpathogens, which can activate the six protein components of the alternative pathway without prior immunization to their specific antigens (Table 2, also from Pangburn). Five of these proteins are unique to the alternative pathway; only C3 is common to both pathways. Since none of these activators requires prior stimulation of antigen-specific antibodies, the alternative pathway offers immediate response to certain serious infections, to amelioration of acute symptoms of dangerous immune complex syndromes, and to amplification of available C3b to play its amboceptor role when a rapid response is required.

The exact mechanism by which the first metastable molecules of C3 are produced is not yet absolutely certain. According to Pangburn, the consensus is that there is spontaneous hydrolysis of the thioester group in native C3, thereby leading to C3b deposition not only on the target pathogen but also indiscriminately on all particles, including the host’s own cells. A system of control factors rapidly inactivates the C3b molecules bound to host cells or other nonactivators, but these control factors work very slowly on C3b bound to particles recognized by the system as proper sites of activation. C3b on such activating particles forms an enzyme that can function like the C3/C5 convertase of the classical pathway. It activates C3 and deposits C3b molecules onto the surface being attacked. An

| Table 1. Activators of the alternative pathway* |
|-------------------------------|---------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| **Pathogens and particles of microbial origin** | **Nonpathogens** | **Functions** | **Molecular weight** | **Serum concentration (µg/mL)** |
| Many strains of gram-negative bacteria | Human IgG, IgA, and IgE in complexes | Attaches covalently after proteolytic activation generates metastable C3b. C3b fragment is part of C3/C5 convertase. | 185,000 | 1200 |
| Lipopolysaccharides from gram-negative bacteria | Rabbit and guinea pig IgG in complexes | Binds to C3b forming the precursor of the C3/C5 convertase (C3b,Bb). Bb subunit of this complex is a serine protease. | 93,000 | 200 |
| Many strains of gram-positive bacteria | Cobra venom | Serine protease that activates factor B when B is in complex with C3b. | 24,000 | 1 |
| Teichoic acid from gram-positive cell walls | Heterologous erythrocytes (rabbit, mouse, chicken) | Regulator (negative) that inactivates the C3/C5 convertase by dissociating its subunits. Also a cofactor for factor 1. | 150,000 | 560 |
| Fungi and yeast cell walls (zymosan) | Anionic polymers (dextran sulfate) | Regulator (negative) that is a serine protease. Inactivates C3b with the aid of factor H or the C3b receptor (CR1). | 88,000 | 34 |
| Some viruses and virus-infected cells | Pure carbohydrates (agarose, insulin) | Regulator (positive) that enhances activation by binding to and stabilizing the C3/C5 convertase. | 224,000 | 20 |

*Reprinted with permission from Pangburn.1

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**Table 2. Proteins of the alternative pathway**

| Symbol | Name | Functions | Molecular weight | Serum concentration (µg/mL) |
|--------|------|-----------|-----------------|-----------------|-----------------|-----------------|-----------------|
| C3     | C3   | Attaches covalently after proteolytic activation generates metastable C3b. C3b fragment is part of C3/C5 convertase. | 185,000 | 1200 |
| B      | Factor B | Binds to C3b forming the precursor of the C3/C5 convertase (C3b,Bb). Bb subunit of this complex is a serine protease. | 93,000 | 200 |
| D      | Factor D | Serine protease that activates factor B when B is in complex with C3b. | 24,000 | 1 |
| H      | Factor H | Regulator (negative) that inactivates the C3/C5 convertase by dissociating its subunits. Also a cofactor for factor 1. | 150,000 | 560 |
| I      | Factor I | Regulator (negative) that is a serine protease. Inactivates C3b with the aid of factor H or the C3b receptor (CR1). | 88,000 | 34 |
| P      | Properdin | Regulator (positive) that enhances activation by binding to and stabilizing the C3/C5 convertase. | 224,000 | 20 |

*Reprinted with permission from Pangburn.1
amplification process now comes into play. Each new C3b bound to this surface can form C3/C5 convertase that can deposit more C3b, and the process repeats itself until the surface is saturated or the supply of complement component is depleted. By this mechanism the alternative pathway of complement activation is capable of depositing more than two million C3b molecules onto the surface of a particle in less than 5 minutes after contact!

The confluence of the classical and alternative pathways is illustrated in Figure 1. This highly simplified diagram emphasized that once the two pathways reached the point where their respective C3 convertases provided C3b to the ongoing system, progression through C5 to C9 activation proceeded on a shared final pathway to completion.

New Technologies Provide Fundamental New Knowledge

It is not within the province of this review to describe details of the elegant exploitation of new technologies for protein purifications, recombinant DNA technology, identification of genetic location, cloning of genes for various complement components, transfection, and production of purified gene products, use of knock-out animal models to verify functional capacities of gene products, and many more.

Research in the complement field accelerated and diversified rapidly during the second half of the 20th century. Table 3 summarizes the International Complement Workshops from 1963 to 1993. The 1963 meeting at the National Institutes of Health in the United States was attended by 25 scientists; 15 manuscripts were eventually published. A meeting in Cambridge in the United Kingdom in 1991 had more than 320 attendees; 320 abstracts were published. In Rother and Till’s book, The Complement System, published in 1988,4 one 16-page chapter on complement components cited 208 references, while a 39-page chapter on genetics and polymorphism of complement components cited 380 references.

Among other accomplishments during this era was identification of individual cells and tissues in which complement components were produced. They proved to be more diverse than expected. While the main site of synthesis was the hepatocytes in the liver, another prolific site was the fimbriae of the Fallopian tubes. In vitro studies showed that a great many cells could make complement components, e.g., myeloid cells, macrophages, intestine, bladder, skin, lungs, and many others.

As understanding of the complement system expanded during the explosion of new knowledge described in an earlier section, an increasing awareness developed of the important benefits humanity reaps from the system as well as the potential for serious negative consequences if all, or parts, of the system break down or function out of control. Individuals born with deficiencies of various complement components may become chronic invalids, their life spans greatly shortened, vulnerable to fulminant infections, and possibly more susceptible to certain malignancies. The complexity of the complement system, its access to every tissue in the body via its

<table>
<thead>
<tr>
<th>Workshop</th>
<th>Date</th>
<th>Location</th>
<th>Number of published abstracts</th>
<th>Reference</th>
</tr>
</thead>
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<tr>
<td>1</td>
<td>February 28–March 1, 1963</td>
<td>National Institutes of Health, Bethesda, MD</td>
<td>Summary only</td>
<td>(44)</td>
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<tr>
<td>2</td>
<td>January 17–19, 1966</td>
<td>La Jolla, CA</td>
<td>30</td>
<td>(45)</td>
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<td>3</td>
<td>June 3–5, 1968</td>
<td>Harvard Medical School, Boston, MA</td>
<td>30</td>
<td>(46)</td>
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<td>4</td>
<td>January 27–29, 1971</td>
<td>Johns Hopkins University, Baltimore, MD</td>
<td>39</td>
<td>(47)</td>
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<tr>
<td>5</td>
<td>February 23–25, 1973</td>
<td>Hotel Del Coronado, Coronado, CA</td>
<td>68</td>
<td>(48)</td>
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<tr>
<td>6</td>
<td>November 23–25, 1975</td>
<td>Sarasota, FL</td>
<td>101</td>
<td>(49)</td>
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<td>7</td>
<td>November 20–22, 1977</td>
<td>St. Petersburg Beach, FL</td>
<td>137</td>
<td>(50)</td>
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<td>8</td>
<td>October 14–16, 1979</td>
<td>Key Biscayne, FL</td>
<td>140</td>
<td>(51)</td>
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<tr>
<td>9</td>
<td>November 22–25, 1981</td>
<td>Key Biscayne, FL</td>
<td>177</td>
<td>(52)</td>
</tr>
<tr>
<td>10</td>
<td>May 25–27, 1983</td>
<td>Mainz, Germany</td>
<td>214</td>
<td>(53)</td>
</tr>
<tr>
<td>11</td>
<td>November 5–8, 1985</td>
<td>Key Biscayne, FL</td>
<td>266</td>
<td>(54)</td>
</tr>
<tr>
<td>12</td>
<td>September 18–21, 1987</td>
<td>Chamonix, France</td>
<td>320</td>
<td>(55)</td>
</tr>
<tr>
<td>13</td>
<td>September 10–15, 1989</td>
<td>San Diego, CA</td>
<td>308</td>
<td>(56)</td>
</tr>
<tr>
<td>14</td>
<td>September 15–20, 1991</td>
<td>Cambridge, UK</td>
<td>320</td>
<td>(57)</td>
</tr>
<tr>
<td>15</td>
<td>September 27–October 1, 1993</td>
<td>Kyoto, Japan (planned)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Reprinted with permission from Weiler (references in table refer to original paper).5
location within the circulatory system, and the extraordinary speed with which it can react to perturbations require clinical investigators to proceed with great caution in efforts to manipulate complement. Well-intentioned efforts to achieve desirable ends must be guided by a full understanding of undesirable consequences that could follow from upsetting the delicate balance that undergirds its vital roles on behalf of human survival.

Regulation of the Complement System

For every stage of activation of the classical pathway there are regulatory proteins which bring about rapid destruction of the activated factors, thereby preventing complete consumption of plasma C4 and C2 in the fluid phase. Rapid inactivation of C1 to prevent uncontrollable activation of complement is brought about by the nonenzymic protein, C1 inhibitor, a heavily glycosylated 100,000-Da protein normally present in plasma at a concentration of 100 to 150 µg/mL. C1 inhibitor combines with a catalytic site on the light chains of C1r and C1s, causing them to dissociate from C1, leaving C1q bound to the target membrane. This sequence of events occurs very rapidly (in 10–20 sec), thus limiting further C1 activation. Bound C42 (the C3/C5 convertase) is eliminated in two phases. First, C2 dissociates spontaneously from C4, a process which is promoted by C4-binding protein and decay-accelerating factor (DAF; CD55), which is found within the membranes of RBCs and many different cell types. In the second phase, residual bound C4b is further degraded into C4c, which dissociates from the target membrane, leaving only C4d attached to the target cell membrane. This degradation is brought about by the enzyme Factor I, aided by C4BP and the C3 receptor CR1. Factor I also cleaves bound C3b. Once C3 is inactivated it can no longer bind C5, hence further production of the membrane attack complex is prevented. This single example of inhibitor-mediated regulation illustrates the intricacy of regulatory processes within the complement area.

At the time of activation of C5, the C5a anaphylatoxin fragment is released, with the effects on neutrophils and monocytes shown in Table 4. Activated C5 attaches to the membrane of the target cell, where it binds stoichiometrically to C6, serving as an acceptor for C7, leading finally to assembly of the completed membrane attack complex (MAC). The MAC inserts itself into the membrane, bringing about lysis of the target cell via the formation of circular “pores” varying from 20 to 100 Å in diameter.

Complement Mediators of Inflammation—the Anaphylatoxins

Among the many protective roles played by the complement system is its mobilization of the body’s inflammatory components in response to infections and to tissue damage from any cause. Key players in this arena are the anaphylatoxins, low-molecular-weight fragments derived from complement factors C3, C4, and C5. All of these polypeptides contribute to spasmogenicity, i.e., they promote smooth muscle contraction and increase vascular permeability. These biological properties have been shown to induce anaphylaxis in certain test animals. The biochemical compositions of these polypeptides are very similar, and one of the anaphylatoxins, C5a, plays a leading role in the host’s inflammatory response. Each of the anaphylatoxins is cleaved from the amino terminus of the alpha chain of its parent molecule by a specific convertase enzyme formed during complement activation. The fragments are designated C3a, C4a, and C5a, and when released into the plasma, C3a and C4a are rapidly converted by the enzyme serum carboxypeptidase to their inert des-Arg analogs. C5a is largely catabolized after internalization by its target cells. Note the complexity of Figure 2 and compare its complexity with the simplicity of Figure 1. The “lectin pathway” has been added to the classical and alternative pathways. Lectins are nonimmune carbohydrate-recognizing molecules found in plants and animals. Two lectins of human origin are conglutinin and mannose-binding protein, shown in Figure 2 as mannose-binding lectin (MBL). For example, MBL can activate the complement system when it binds to mannose receptors on certain bacterial and virus surfaces. Yet even the pathway depicted in Figure 2 is simpler than reality because it does not

Table 4. Granulocyte-related activities of human C5a*

<table>
<thead>
<tr>
<th>Target cells</th>
<th>Cellular responses</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neutrophils</td>
<td>Augmented adherence</td>
</tr>
<tr>
<td>Monocytes</td>
<td>Cellular polarization</td>
</tr>
<tr>
<td></td>
<td>Chemotactic migration</td>
</tr>
<tr>
<td></td>
<td>Degradation and enzyme release</td>
</tr>
<tr>
<td></td>
<td>Production of toxic oxygen metabolites</td>
</tr>
<tr>
<td></td>
<td>Enhanced arachidonic acid metabolism</td>
</tr>
<tr>
<td></td>
<td>Augmented expression of complement receptors</td>
</tr>
<tr>
<td></td>
<td>Increased elaboration of IL-1 by monocytoploid cells</td>
</tr>
</tbody>
</table>

*Reprinted with permission from Chenoweth.
History of complement system

include many other complement inhibitors and the receptors for many complement factors and byproducts.

The biological activities of the anaphylatoxins are illustrated in Tables 4 and 5. It is clear from Table 5 that the spasmogenic properties (smooth muscle contraction and vascular permeability) of C5a are far more potent than those of C3a and C4a. The same predominance of C5a over C3a and C4a was observed for the granulocyte- and monocyte-related activities listed in Table 4, justifying the reputation of C5a as a major anaphylatoxin inflammatory mediator. As admirable as C5a’s superiority appears in this general context, Chenoweth\(^5\) cautions that it must be recognized that C5a may also act systemically and trigger similar types of granulocytic responses in blood or distant organs. For example, when this occurs during complement-induced pulmonary vascular leukosequestration, C5a-activated granulocytes release cytotoxic substances that destroy normal tissues. In this case, and in certain autoimmune disorders, normal host defense mechanisms may actually contribute to the pathogenesis of a specific disease, such as adult respiratory distress syndrome or rheumatoid arthritis.

C5a initiates a wide variety of responses (Table 4) via binding to receptors that are known to be located on target cells (peripheral blood neutrophils and monocytes, alveolar macrophages, and cultured cells of monocytoid origin). Characterization of these receptors utilized radio-iodinated (\(^{125}\)IC5a) or fluorescein-labeled human C5a (fl-C5a). The C5a receptors of the previously described cells and the plasma membrane fraction isolated from these cells are functionally indistinguishable.

At the time Chenoweth was writing, other investigators were attempting to define more precisely the “recognition domain” determinants of C5a because peptide or chemical analogs of the regions could possibly act as competitive antagonists to C5a binding and thus function as anti-inflammatory compounds. Work in progress included similarly directed studies of receptors for CR2, CR3, CR4, H-R, C1q-R, C3a/C4a-R, and CR3e-R. Also under way were similar studies of opsonic IgG, opsonic C3, opsonic C4, and fibronectin. Other studies were being directed at CR1 membrane complement receptors on human, sheep, rabbit, mouse, rat, and guinea pig RBCs. Identification and characterization of complement receptors on neutrophils, monocytes, lymphocytes, Kupfer cells, phagocytes, and platelets goes on apace.
Relation of Complement Components to Blood Group Serology

Not to be outdone by the flourishing worldwide community of blood group specialists, complementologists became card-carrying blood group specialists themselves with the announcement in 1978 by O’Neill et al. that the Chido/Rodgers blood group antigens were in fact distinct antigenic components of human complement C4. The subject is well chronicled in Blood Transfusion in Clinical Medicine by Mollison, Engelfriet, and Contreras. Since the antigens are generally detectable in normal human plasma, it is assumed that their presence on RBC membranes reflects adsorption from the individual’s plasma. The Ch and Rg determinants are localized to chromosome 6. Anti-Ch and anti-Rg are found only in Ch- or Rg-negative subjects. RBCs of the relatively rare C4 null patients are Ch- and Rg-negative. Fortunately, incompatibility within the Ch/Rg system has not caused documented hemolytic transfusion reactions or HDN, although there have been rare reports of anaphylactic symptoms in multi-transfused Ch- or Rg-negative recipients of platelet concentrates or plasma transfusions.

As a more contemporary example of the complement system’s encroachment on the blood bank serologist’s turf, elucidation of the clinical role of the decay-accelerating factor (DAF;CD55) glycoprotein associated with the Cromer blood group system has made important progress in recent years. Like the Ch/Rg antigens, the Cromer antigens are widely detectable on multiple human cells: RBCs, WBCs, platelets, and endothelial and epithelial tissues. Lublin’s group at Washington University in St. Louis has recently completed a detailed definition of the molecular basis of nine Cromer antigens. The Cromer system antigens have been shown to reside on the DAF protein, one of the regulators of the complement system and hence one of the protectors of autologous cells and tissues from complement-mediated damage. Antibodies to Cromer system antigens could lead to RBC destruction, but hemolytic transfusion reactions and HDN have not been reported. However, it is necessary to identify antibodies in the Cromer system during pretransfusion testing.

One can expect a major expansion of collaborative research in the overlapping areas of complement physiology and transfusion medicine.

The Role of Complement in Immune Destruction of Transfused RBCs and in Autoimmune Hemolytic Anemias

Thus far, this review has focused on the role of complement in protection against infectious diseases. However, it has also shown that there is a downside to the complement story.

For many decades, the human RBC has been well known as a victim of complement activity, as exemplified by complement’s noxious contribution to hemolytic transfusion reactions and its frequent role in autoimmune hemolytic anemias (AIHAs). In the diagnosis and management of both of these life-threatening illnesses, it has challenged the skills of laboratory technicians, manufacturers of specific antiglobulin reagents, and molecular biology researchers. Complement can be activated by naturally occurring antibodies (e.g., anti-A and anti-B in normal group O, A, and B blood donors) as well as by blood group alloantibodies formed in response to multiple transfusions or pregnancies (e.g., antibodies to antigens within the Kidd, Duffy, and Kell systems). In these circumstances, blood group alloantibodies that activate complement can provoke more serious hemolytic reactions, sometimes with rapid intravascular RBC destruction and accompanying hemoglobinuria.

Complement can play an analogous role in many AIHAs (e.g., the biphasic anti-P Donath-Landsteiner autohemolysin in paroxysmal cold hemoglobinuria). Complement’s role is supported by the frequent finding of strong reactivity with anti-C3d reagents accompanying strong reactivity with anti-IgG reagents on the RBCs of some patients with severe AIHAs.

The extensive literature on the role of complement in immune destruction of human RBCs is well reviewed in Blood Transfusion in Clinical Medicine by Mollison, Engelfriet, and Contreras.
The Role of Complement in the Rheumatic Diseases

As noted earlier, the “normal” functions of the complement system may sometimes become a double-edged sword. Autoantibodies and circulating immune complexes in certain rheumatic diseases may activate the complement system, thereby enhancing complement’s “good” pro-inflammatory response but leading to further tissue destruction. The same undesired effect may lead to deposition of immune complexes in the walls of blood vessels (vasculitis), resulting in necrosis of vessels. When immune complex vasculitis occurs in the kidneys, lungs, skin, or other organs, patients may become seriously ill.

Therapeutic complement inhibitor approaches have been considered for treatment of bullous pemphigus, rejection of transplanted tissues, Alzheimer's disease (because plaques contain high levels of classical and alternative pathway components as well as MAC components), immune-based fetal loss, HIV, and a great many other serious medical conditions. Other disorders that interface importantly with the complement system (including systemic lupus erythematosus, rheumatoid and related arthritides, and cryoglobulinemia) are well reviewed by Atkinson et al.10

Looking ahead, careful attention is being given to important questions that need further exploration. Where in the three activation pathways (classical, alternative, lectin) should the various inhibitors be applied: prior to or coincident with the shared C3b step? Can local, systemic, or both therapeutic strategies be predictably and safely controlled? Should research emphasis focus on recombinant or nonrecombinant agents? What important proteins outside the complement system (e.g., clotting factors) could also be inhibited unintentionally? How would the effectiveness of complement inhibitors stack up against other pro-inflammatory measures? There are several major concerns regarding all of the above issues: what would be the impact of each with respect to increased risks of autoimmunization,11 and how might each alter the self-tolerance of B cells?12

The Role of Complement in Paroxysmal Nocturnal Hemoglobinuria

The rapid broadening of understanding of the complement system has promoted improved understanding of the pathogenesis of many diseases, both common (e.g., rheumatoid arthritis) and rare (e.g., paroxysmal nocturnal hemoglobinuria [PNH]). New understanding has given rise to imaginative complement research designed to improve the lives of individuals suffering from these ailments. As with any innovative development at the start, enthusiastic predictions and high expectations abound. While unanticipated difficulties may delay or even derail some of these efforts, some important goals have been achieved.

PNH is an example of a highly successful laboratory and clinical research effort. A recent article in the New England Journal of Medicine13 describes a study based in Leeds, England, on eleven transfusion-dependent adult patients (six men and five women) with well-documented PNH, a disorder arising as a result of a somatic mutation of the PIG-A gene in a hematopoietic stem cell. The mutation results in the subsequent production of blood cells with a deficiency of the DAF proteins that protect normal RBCs against attack by the complement system. In a well-designed, well-standardized, and well-executed 12-week clinical and laboratory study, the effects of intravenous infusions of eculizumab, a humanized antibody that inhibits the activation of terminal complement components C5 to C9, were examined. If effective, the drug would be expected to reduce RBC hemolysis sufficiently to eliminate the hemoglobinuria, reduce or eliminate the transfusion dependency, and relieve troublesome symptoms related to the chronic hemolysis. Well-chosen laboratory tests to quantitate the rate of hemolysis were performed at regular intervals, as was quantitation of the numbers of abnormal RBCs in the circulation and visual daily quantitation of the urine for detectable hemoglobinuria. The standardized interview of the European Organization for Research and Treatment of Cancer (EORTC), titled the QLQ-30, was employed periodically to evaluate quality of life.

Patient compliance was excellent. No evidence of formation of antibodies to the eculizumab was found, and no untoward drug-related side effects were detected. Laboratory results documented significant reduction in the rate of hemolysis (though not total normalization, and the Hb level did not rise). Paroxysms of hemoglobinuria were markedly decreased. Regarding quality of life, there was significant improvement in the domains of global health (p = 0.02), physical functioning (p < 0.001), emotional functioning (p < 0.001), cognitive functioning (p = 0.002), fatigue (p < 0.001), dyspnea (p = 0.002), and insomnia (p = 0.049).
All the patients enlisted in a 12-month extension of the study, with no ill effects noted and with sustained improvement in their symptoms and laboratory studies. The authors conclude: “Eculizumab appears to enhance the survival of type III PNH erythrocytes, improving the quality of life and reducing the extent of hemolysis, hemoglobinuria (the clinical hallmark of PNH), and the need for blood transfusions in patients with PNH. This study confirms that terminal complement activation is the key mediator of erythrocyte destruction in PNH.”

The author of the present review strongly encourages readers to enjoy this exemplary publication for its lucidity and inspirational value.

The Emerging Role of Complement Inhibitors in Immunohematology

The PNH article cited in the previous section describes primarily a clinical trial of a candidate drug to add to the therapeutic armamentarium of transfusion medicine. As a fitting finale to the present historical review of the complement system, I would like to summarize briefly an ongoing “work in progress” at the New York Blood Center by a research group under the direction of Karina Yazdanbakhsh. The titles of three recent publications from her laboratory track the program’s progress during the past 3 to 4 years. The title of the first paper, published in 2003, is “Complement receptor 1 inhibitors for prevention of immune-mediated red cell destruction: potential use in transfusion therapy.” The paper describes preparation of recombinant human CR1 from which several truncated soluble CR1 proteins were expressed and tested for their complement inhibitory properties. Two of the derivatives were selected for in vivo inhibition of complement-mediated immune hemolysis in a xenotransfusion mouse model and for lowering the deposition of C3 and C4 on the target RBCs. The second paper, published in 2004 and titled “Prevention of complement-mediated immune hemolysis by a small-molecule compound,” describes the screening of a commercial 10,000 compound library enriched in anti-inflammatory molecules, from which a single 549-Da compound was selected on the basis of its ability to reduce hemolysis induced via the classical and alternative pathways. In a xenotransfusion mouse model, significantly prolonged RBC survival was observed, as was reduced C3 deposition on the surviving RBCs. The findings support the possibility that the small-molecule complement compound could be useful in a human transfusion setting. The third publication, “Characterization of complement receptor-1 domains for prevention of complement-mediated red cell destruction,” was published in 2005. This paper, building on the findings of the two preceding papers, chronicles further the search for even smaller soluble CR1 inhibitors. A 250-amino acid region in CR1 that possessed antihemolytic activity and was effective at prolonging survival of transfused RBCs was identified. Mutation of two critical residues in this 250-amino acid domain resulted in improved complement-inhibitory functions compared to the unmutated CR1 derivatives, resulting in a more potent inhibition of complement activation in vitro. However, in vivo, the activity of the mutant protein was comparable to the wild type molecules. These studies indicated the importance of testing CR1 inhibitors in vivo.

Taken together, the three careful and sophisticated studies indicate the increased precision achievable when using today’s scientific technology to explore structure/function relationships in evaluation of complement inhibitors.

The Index Medicus lists more than 40,000 publications under “complement” in the past 39 years. Complement system research continues to grow. Enjoy it!

Acknowledgment

The author expresses special appreciation to colleagues Prof. John P. Atkinson and Sir Peter J. Lachmann for their wise advice and welcome encouragement.

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Hugh Chaplin Jr. MD, Emeritus Professor of Medicine and Pathology, Washington University School of Medicine, St. Louis, Missouri.
On a much higher than reported incidence of anti-c in R₁R₁ patients with anti-E

W.J. JUDD, L.R. DAKE, AND R.D. DAVENPORT

A previous study involving tube IATs, untreated RBCs, and a low-ionic-strength additive reagent revealed that approximately one-third of R,R₁ patients with anti-E have a concomitant anti-c. However, the current study finds a much higher incidence of anti-c in such patients, using gel technology in conjunction with ficin-pretreated RBCs. Results of antibody identification studies and transfusion records of 82 R,R₁ patients with anti-E were reviewed. Serologic test methods included a LISS wash solution for tube IATs (15 min at 37°C, anti-IgG), ficin-tube IATs (30 min at 37°C, anti-IgG + anti-C3), and gel IATs (untreated or ficin-treated RBCs or both, anti-IgG gels). LISS-tube or gel IATs with untreated RBCs revealed anti-c in 32 patients with anti-E. When gel-IAT and ficin-pretreated RBCs were used, 21 additional patients with anti-E were found to have anti-c. In samples from 26 R,R₁ patients with anti-E, anti-c was not demonstrable by ficin-gel IATs, and in 3 cases, the ficin-gel tests were inconclusive. In five cases in which E- RBCs not tested for c antigen were transfused to patients found by ficin-gel IAT to be without anti-c, all subsequently performed crossmatches with E- c- untested RBCs were compatible. The incidence of anti-c in R,R₁ patients with anti-E in this study was 32 of 82 (39%) with untreated RBCs and 53 of 82 (65%) when the ficin gel data were included. The latter is significantly higher than the 32 percent incidence previously reported (p = 0.0001). Accordingly, all patients at our facility with an Rh antibody are now tested for those additional Rh antibodies they can make, as predicted from their Rh phenotype. The data from this study strongly support the selection of c- RBCs for this patient population.

Key Words: anti-c, anti-E

The immune response to RhCE protein is variable. For example, R₁R₁ (c-, E−) individuals may make anti-c, anti-E, anti-c plus anti-E, or anti-cE (Rh 27), the latter reacting with a determinant encoded by c and E within the same haplotype (in cis). Some individuals may make all three antibodies, as demonstrated by adsorption-elution studies, or a combination of anti-c plus anti-E.

Shirey et al. found the incidence of anti-c in R₁R₁ patients with anti-E to be 32 percent when performing IATs by a low-ionic-strength additive technique. They also showed if the RBCs selected for transfusion were E- but not tested for c antigen, 18.5 percent of recipients developed anti-c. Since their report, gel technology has emerged and has been found to have good sensitivity for Rh antibodies. Further, we have shown that tests performed with anti-IgG gel cards and ficin-pretreated RBCs are exquisitely sensitive for Rh antibodies.

In this report we present data from ficin-gel tests that demonstrate a higher than previously published incidence of anti-c in R₁R₁ patients with anti-E. These data support the selection of c- RBCs for this patient population.

Materials and Methods

ID-MTS gel technology was from Ortho-Clinical Diagnostics (Raritan, NJ). Reagent RBCs, both untreated and ficin-treated, and LISS (Löw and Messeter formulation) were from ImmucorGamma, Norcross, Georgia. For gel testing, untreated reagent RBCs were prepared in ID-MTS Diluent 2 at a concentration of 0.8% and tested on anti-IgG cards according to the manufacturer's product circular. Gel tests with ficin-pretreated RBCs were similarly performed on anti-IgG cards; such testing (previously validated by us) conflicts with the manufacturer's product circular, which stipulates the use of buffered gel cards. LISS-tube tests were incubated at 37°C for 15 minutes, washed four times with saline, and tested with anti-IgG (Ortho). Ficin-tube tests were incubated at 37°C for 30 minutes and tested with polyspecific (anti-IgG + C3) antoglobulin reagent (Ortho). Negative tube tests were validated with IgG-coated RBCs (ImmucorGamma).

Serologic and transfusion records of 82 R₁R₁ patients with anti-E were reviewed. No further testing was performed on 32 patients with concomitant anti-c by routine testing (LISS-tube and ficin-tube). Ficin-gel tests were used to detect the presence or absence of anti-c in samples from the remaining 50 patients.
Results
We found anti-c in 53 out of 82 (65%) R_1R_1 patients with anti-E (Table 1). In 32 cases, the presence of anti-c was evident from the results of LISS-tube or gel IATs. In the other 21 cases (see Fig. 1 for an example), the presence of anti-c was clearly demonstrable only in ficin-gel IATs. However, among these 21 cases, weak reactivity was also seen in two cases with some untreated c+, E– RBCs in gel IATs and suspected from the results of ficin-tube tests in five cases. There were three additional cases in which the presence of anti-c could not be determined due to panreactivity in ficin-gel tests.

Of the 26 R_1R_1 patients with anti-E that did not have anti-c clearly demonstrable by ficin gel, 18 did not require transfusion (five were pregnant, and there were records of previous transfusions on six). A further eight patients were transfused with E– RBCs that were not tested for c antigen. Three of these patients were lost to follow-up. We had the opportunity to test the five remaining patients for anti-c in subsequent antiglobulin crossmatches with E– donor units that had not been selected to be c–; in fact, two patients were transfused with Rh– RBCs that were undoubtedly c+. These crossmatches were performed between 3 and 20 months after anti-E was initially detected in the patients’ plasma. All units (n = 10) were crossmatch compatible.

Discussion
The development of alloantibodies to RBC antigens through transfusion or pregnancy is not benign. Patients who become alloimmunized are at risk of hemolytic transfusion reactions and high-risk pregnancies associated with HDN. Further, alloimmunization is sometimes accompanied by autoantibody formation, which may lead to autoimmune hemolytic anemia. These risks prompted some investigators to recommend the use of phenotypically matched RBCs, especially for sickle cell anemia patients. As shown recently, this recommendation is by no means universally followed.

In the 11th edition of the AABB Technical Manual, it was suggested that R_1R_1 transfusion candidates who have made anti-E should be transfused with R_1R_1 RBCs to prevent formation of anti-c, possible posttransfusion hemolysis, and autoantibody formation. This suggestion prompted Shirey and colleagues to determine the incidence of anti-c in 100 R_1R_1 patients with anti-E. In their study, using LISS-tube IATs, they found the incidence to be 32 percent. Among the 68 R_1R_1 patients with anti-E alone, 27 were transfused with E– RBCs that were not typed for c antigen; five (18.5%) of these patients subsequently formed anti-c.

Given our past experiences with gel technology, which is exquisitely sensitive for Rh antibodies, we expected to find a somewhat higher incidence of anti-c in R_1R_1 patients with anti-E than was reported by Shirey et al. However, we did not expect to find a twofold increase (32% vs. 65%; p = 0.0001) through the use of gel technology and ficin-treated RBCs, given that before implementation of gel technology in our laboratory our routine antibody identification protocol included ficin-tube IATs. We postulate that these anti-c antibodies are, for the most part, low-affinity antibodies
that dissociate during the washing phase of tube IATs; gel technology is, of course, a no-wash IAT.

Although we saw no evidence of anti-c development in five R₁R₁ patients with anti-E, as demonstrated by compatible IAT crossmatches with E-, c-untested RBCs, we now select c- blood for R₁R₁ patients with anti-E, regardless of whether or not anti-c is detected. However, due to the comparative rarity of R₂R₂ donors, we do not automatically select R₂R₂ RBCs for patients with anti-C who are c-. Rather, we test for anti-e by ficin gel, if it is not evident by routine studies, and, if it is present, we crossmatch R₂R₂ donor RBCs. We have a similar policy for R₀ patients with anti-C or anti-E, inasmuch as we select C-, E- RBCs for crossmatching only if ficin-gel tests reveal that both anti-C and anti-E are present (Table 2).

Table 2. Policy for ficin-gel testing and blood selection in D+ patients with Rh alloantibodies

<table>
<thead>
<tr>
<th>Patient’s RBCs</th>
<th>If*</th>
<th>Then†</th>
<th>Or, if*</th>
<th>Then†</th>
</tr>
</thead>
<tbody>
<tr>
<td>R₁R₁ anti-E</td>
<td></td>
<td>issue c-, E- RBCs</td>
<td></td>
<td></td>
</tr>
<tr>
<td>R₂R₂ anti-C</td>
<td></td>
<td>exclude anti-e anti-e</td>
<td>issue C-, e- RBCs</td>
<td></td>
</tr>
<tr>
<td>R₀ anti-C</td>
<td></td>
<td>exclude anti-E anti-E</td>
<td>exclude anti-C</td>
<td></td>
</tr>
</tbody>
</table>

*Antibody found in serum with untreated RBCs.
†Exclusion tests performed with ficin-treated RBCs by gel technology.

References

W. John Judd, FIBMS, MIBiol; Louann R. Dake, MA, MT(ASCP)SBB, and Robertson D. Davenport, MD, Department of Pathology, UH-2G332, University of Michigan Medical Center, 1500 East Medical Center Drive, Ann Arbor, MI 48109-0054.
Case report: massive postpartum transfusion of Jr(a+) red cells in the presence of anti-Jrα

Jrα is a high-prevalence antigen. The rare Jr(α−) individuals can form anti-Jrα after exposure to the Jrα antigen through transfusion or pregnancy. The clinical significance of anti-Jrα is not well established. This study reports a case of a 31-year-old woman with a previously identified anti-Jrα who required massive transfusion of RBCs after developing life-threatening postpartum disseminated intravascular coagulopathy. Despite the emergent transfusion of 15 units of Jrα unrelated RBCs, she did not develop laboratory or clinical evidence of acute hemolysis. The patient's anti-Jrα had a pretransfusion titer of 4 and a monocyte monolayer assay (MMA) reactivity of 68.5% (reactivity > 5% is considered capable of shortening the survival of incompatible RBCs). The titer increased fourfold to 64 and the MMA reactivity was 72.5% on Day 10 posttransfusion. Review of laboratory data showed evidence of a mild delayed hemolytic transfusion reaction by Day 10 posttransfusion. Despite rare reports of hemolytic transfusion reactions due to anti-Jrα in the literature, most cases, including this one, reported that this antibody is clinically insignificant or causes only mild delayed hemolysis. Clinicians should be advised to balance the risks of withholding transfusion with the small chance of significant hemolysis after transfusion of Jr(a+) RBCs in the presence of anti-Jrα. Immunohematology 2005;21:97–101.

Key Words: anti-Jrα, massive transfusion, hemolytic transfusion reaction

Jrα is a high-prevalence antigen in all populations. The Japanese population has the highest frequency of the Jr(α−) phenotype. However, even in that population, the incidence is reported to be only 0.03 to 0.12 percent.1 Because of its rarity, the clinical significance of anti-Jrα is not well established. Reid and Lomas-Francis2 describe anti-Jrα as being capable of causing decreased RBC survival. In the obstetric setting, the authors note that although the DAT can be positive, anti-Jrα is not associated with HDN.

Jr(α+) RBCs have been transfused to individuals with anti-Jrα without incident.3–5 On the other hand, there exist in the literature a dozen or so reports highlighting the ability of anti-Jrα to cause both mild to moderate HDN and delayed hemolytic transfusion reactions.6 The typical hemolytic reaction associated with anti-Jrα is delayed, extravascular, and self-limited, and the decreasing Hct is usually accompanied by transiently increased antibody titers.7 More recently, Kwon et al.8 reported a case of anti-Jrα causing acute hemolysis. In this report, we describe the case of a patient with anti-Jrα who developed postpartum disseminated intravascular coagulopathy (DIC) and received 15 units of incompatible Jr(a+) RBCs. Subsequently she had no overt clinical signs of hemolysis, and laboratory findings suggested only mild delayed hemolysis.

Case Report

A 31-year-old multiparous Vietnamese woman with history of three previously uncomplicated pregnancies (with a different partner from the current pregnancy) presented for prenatal care at an outside facility in the 32nd week of her fourth pregnancy. ABO/D typing showed the patient's RBCs to be Group A, D+. The antibody screen test showed that the patient's serum reacted weakly with all reagent RBCs in the antiglobulin phase of testing. RBC treatment using either DTT or ficin had no effect on reactivity. The autologous control and DAT were negative; thus an antibody directed at a high-prevalence antigen was suspected. The patient's serum was then tested with several RBC samples lacking various high-prevalence antigens, including k, Kpβ, and Ch, and RBCs with very weak U expression. All RBCs tested reacted with the patient's serum. The specimen was subsequently referred to our laboratory, where phenotyping of the patient's RBCs for high-prevalence antigens showed that her RBCs possessed Vel, Diβ, k, and Kpβ antigens but lacked Jrα antigens. Jr(α−) RBCs from two different donors located through Serum Cells and Rare Fluids Exchange (SCARF) were not agglutinated by the patient's serum, which was consistent with the presence of anti-Jrα. Allogeneic adsorptions were
performed with W.A.R.M-treated RBCs (W.A.R.M.; Immucor Inc., Norcross, GA) that were also phenotypically matched for Rh and Kidd antigens. No additional alloantibodies were detected in the serum. The anti-Jr\(^{a}\) was shown to have a titer of 4. Because of the difficulty in locating Jr(a–) RBCs, and the possibility of shortened survival of transfused Jr(a+) RBCs, we recommended autologous donation or screening family members in the hope of finding a compatible donor if there was a reasonable concern that the patient or the baby might need transfusion during the pregnancy and delivery.

The patient went into labor during the 38th week of her pregnancy and vaginally delivered a healthy male infant without evidence of HDN. Her postpartum course was complicated by development of DIC and profuse, life-threatening vaginal bleeding, which necessitated an emergent abdominal hysterectomy. Intraoperatively, she received four units of uncross-matched Group O,D– RBCs and 11 units of crossmatch-incompatible Group A, D+ RBCs. Because of the high incidence of Jr\(^{a}\), all of the RBC units were presumably Jr(a+). Her hemorrhage was eventually brought under control after the surgery. Clinically, there was no evidence of acute intravascular hemolysis during the resuscitation effort.

Approximately 15 days after the initial surgery, the patient was found to have developed abdominal abscesses. Because surgery with possible significant blood loss was anticipated, and because of the case report from Kwon et al.\(^6\) reporting acute hemolysis after repeated exposures to Jr(a+) RBCs, two frozen units of Jr(a–) RBCs were imported from Blood Bank of Hawaii. Of note, the only other possible source of units of Jr(a–) RBCs at that time was from Japan. However, these units would not have been immediately available and would not have been fully tested for transfusion-transmissible diseases according to FDA guidelines. Initial attempts at computerized tomography–guided drainage of the abscesses were complicated by perforation of the colon. This necessitated a laparotomy for hemicolecotomy and abscess drainage. Both units of Jr(a–) RBCs were transfused intraoperatively without incident. The patient did not require further transfusions. Twelve days after the second surgery, her Hct was stable at 36.8%.

**Materials and Methods**

Antibody detection and identification were performed by tube method. Antibody detection tests were performed using reagent RBCs (Immucor, Inc.). Antibody identification was performed using panels of reagent RBCs (Ortho-Clinical Diagnostics, Inc., Raritan, NJ, and Immucor, Inc.). Panels of ficin-treated RBCs (Immucor, Inc.) were also used. DTT-treated RBCs were prepared using 0.2 M solution (DTT, Sigma-Aldrich Corp., St Louis, MO). Alloadsorption was performed using W.A.R.M.-treated RBCs (W.A.R.M.; Immucor, Inc.), which were also phenotypically matched for Rh and Kidd antigens.

Polyspecific and monospecific DATs were performed (Bioclone Anti-IgG, Bioclone Anti-C\(_{3d}\), and Biocline AHG, Ortho-Clinical Diagnostics) by standard tube method. The elution was performed using the rapid acid method (Elukit II, Gamma Biologicals, Inc., Houston, TX). Two different enhancement media (PEG, Gamma Biologicals, and O.A.E.S., Ortho-Clinical Diagnostics) were used to enhance antibody agglutination. Antibody titer was determined with serial dilutions of patient's serum using IAT.

Patient’s RBCs were phenotyped using conventional methods for the common antigens. Rare antisera and reagent RBCs were prepared in-house or provided through SCARF. Monocyte monolayer assay (MMA) was performed by the American Red Cross Blood Services of Southern California Region, Los Angeles, California. The method was described in detail elsewhere.\(^8\)

**Results**

Anti-Jr\(^{a}\) was not detectable in the serum immediately after the transfusion of the 15 units of uncrossmatched (presumably Jr\([a+]) RBCs. This was not surprising because of the large volume of crystalloid solutions and blood components administered during the resuscitation. The DAT using polyspecific antihuman globulin at this time was weakly positive (1+), but the patient’s RBCs were negative in the DAT using anti-C\(_{3d}\) or anti-IgG. An eluate was performed and tested and found to contain anti-Jr\(^{a}\). Allogeneic absorption of the eluate, using W.A.R.M-treated RBCs that were also phenotypically matched for Rh and Kidd, did not show any additional alloantibodies.

Because of the concern for a significant delayed hemolytic transfusion reaction, we recommended that the clinicians give intravenous fluids as necessary to maintain adequate renal perfusion and monitor laboratory values, including Hct, bilirubin, LDH, and haptoglobin. Additionally, a sample was sent daily to our reference laboratory for DATs and evaluation for
Massive transfusion in presence of anti-Jr

hemolysis. The patient’s Hct decreased from 38.6% on Day 5 posttransfusion to 30.9% on Day 10. Of note, during this period the patient also developed syndrome of inappropriate antidiuretic hormone secretion and hyponatremia. The resulting fluid retention and dilutional anemia contributed to the decreasing Hct and made it unlikely that it was solely due to hemolysis. On Day 10 posttransfusion, the total bilirubin increased from 0.4 mg/dL at the time of transfusion to 1.6 mg/dL (normal range 0.2–1.0 mg/dL) and LDH increased from 307 to 431 IU/L (normal range 91–180 IU/L). Haptoglobin levels were obtained only on Days 4 and 5 posttransfusion; both were well within normal range. Anti-Jr became detectable in the serum again on Day 6 posttransfusion and the titer had risen from a pretransfusion titer of 4 to 64. Concurrently, the reactivity of the polyspecific DAT increased from 1+ to 2+, and both the monospecific DATs (using anti-IgG and anti-C3) reacted 2+ at 6 days posttransfusion. Both the prenatal pretransfusion serum sample and a serum sample obtained on Day 10 posttransfusion were sent for MMA testing, which showed 68.5% and 72.5% reactivity respectively (reactivity > 5% is considered capable of shortening the survival of incompatible RBCs). However, visual check of serial serum samples showed no evidence of hemolysis, and the patient continued to have no overt clinical evidence of intravascular hemolysis during the posttransfusion period. Select laboratory values are shown in Table 1.

<p>| Table 1. Results of select pertinent laboratory tests pretransfusion and in the days after massive transfusion of 15 units of presumably Jr(a+) RBCs in the presence of anti-Jr. |
|---|---|---|---|---|---|---|---|</p>
<table>
<thead>
<tr>
<th>Day (posttransfusion)</th>
<th>Hct (%)</th>
<th>Na (135–145 mmol/L)*</th>
<th>LDH (91–180 IU/L)*</th>
<th>Total bilirubin (0.2–1.0 mg/dL)*</th>
<th>DAT</th>
<th>Anti-Jr* titer</th>
<th>Other lab values</th>
</tr>
</thead>
<tbody>
<tr>
<td>-30 (pretransfusion)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Negative</td>
<td>4</td>
<td>MMA reactivity: 68.5% (≥ 5%)*</td>
</tr>
<tr>
<td>0</td>
<td>24</td>
<td>151</td>
<td>307</td>
<td>0.4</td>
<td>-</td>
<td>-</td>
<td>Prothrombin time = 36.5, International normalized ratio = 3.53, Partial prothrombin time = 138.8</td>
</tr>
<tr>
<td>50.7</td>
<td>7.4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Fibrinogen = 72 mg/dL</td>
</tr>
<tr>
<td>1</td>
<td>33.2</td>
<td>139</td>
<td>-</td>
<td>0.9</td>
<td>Polyspecific: 1w anti-IgG: 0 anti-C3: 0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>33.4</td>
<td>138</td>
<td>261</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Haptoglobin 121 (43–212 mg/dL)*</td>
</tr>
<tr>
<td>5</td>
<td>38.6</td>
<td>125</td>
<td>118</td>
<td>125</td>
<td>Polyspecific: 2+ anti-IgG: 2+ anti-C3: weak</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>6†</td>
<td>38.2</td>
<td>123</td>
<td>391</td>
<td>-</td>
<td>Polyspecific: 2+ anti-IgG: 2+ anti-C3: 2+</td>
<td>64</td>
<td>-</td>
</tr>
<tr>
<td>8†</td>
<td>33.6</td>
<td>132</td>
<td>386</td>
<td>2.2</td>
<td>Direct 0.5</td>
<td>Polyspecific: 1+ anti-IgG: 1+ anti-C3: 1+</td>
<td>64</td>
</tr>
<tr>
<td>10†</td>
<td>30.9</td>
<td>132</td>
<td>431</td>
<td>1.6</td>
<td>Polyspecific: 2+mf anti-IgG: 1+mf anti-C3: 1w mf</td>
<td>64</td>
<td>MMA reactivity: 72.5% (≥ 5%)*</td>
</tr>
<tr>
<td>15†</td>
<td>28.8</td>
<td>133</td>
<td>-</td>
<td>-</td>
<td>Polyspecific: 2+ anti-IgG: 1+ anti-C3: 2+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>17</td>
<td>25.2</td>
<td>132</td>
<td>372</td>
<td>0.7</td>
<td>Direct 0.1 (0.0–0.2 mg/dL)*</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>20</td>
<td>30.0</td>
<td>128</td>
<td>-</td>
<td>0.6</td>
<td>-</td>
<td>-</td>
<td>Haptoglobin 201 (43–212 mg/dL)*</td>
</tr>
<tr>
<td>27</td>
<td>36.8</td>
<td>134</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Urine bilirubin negative</td>
</tr>
</tbody>
</table>

*Normal range values
†Results from Days 6 to 10 show evidence of onset of mild delayed hemolysis. Visual checks of serum showed no evidence of hemolysis through this period.
‡On Day 15, patient underwent abdominal surgery and received two units of Jr(a–) RBCs intraoperatively.
Discussion

Because of the rarity of anti-Jr\textsuperscript{a}, its clinical significance is not clear. Several reports of anti-Jr\textsuperscript{a} causing mild to moderate HDN\textsuperscript{9,10} exist in the literature, but others have questioned the validity of the diagnoses in such cases.\textsuperscript{11} Mild to moderate delayed hemolytic transfusion reactions\textsuperscript{9,12,13} have also been reported in association with anti-Jr\textsuperscript{a}. More recently, Kwon et al.\textsuperscript{6} reported a concerning case of acute hemolytic transfusion reaction due to this antibody in the setting of repeated exposures to the antigen within a week. On the other hand, others have reported no clinical evidence of hemolysis after incompatible transfusions, even in the face of rising antibody titers.\textsuperscript{4} Studies using monocyte phagocytosis\textsuperscript{14} and MMA\textsuperscript{8,15} suggested that most anti-Jr\textsuperscript{a} are not clinically significant. The lack of clinical significance in most cases could be in part attributed to the low antigen density of Jr\textsuperscript{a} on the RBC membrane.\textsuperscript{14,16}

Additional data are needed to fully establish the clinical significance of anti-Jr\textsuperscript{a}. The MMA has been proposed as a valuable tool for predicting the clinical significance of antibodies to high-prevalence antigens such as anti-Jr\textsuperscript{a}.\textsuperscript{8,15} A recent retrospective review of 20 years of MMA data by Arndt and Garratty\textsuperscript{8} showed that only results greater than 5\% are potentially clinically significant. Furthermore, one-third of patients with MMA results between 5.1\% and 20\% had clinical signs of hemolysis (defined as jaundice, fever, chills, change in blood pressure, back pain, vomiting, tachypnea, and hemoglobinuria\textsuperscript{8}), and two-thirds of patients with MMA results greater than 20\% reactivity had such symptoms. When clinical signs of hemolysis were absent, patients from both groups often had laboratory indications of hemolysis, which included decreased Hb, Hct, and haptoglobin, increased bilirubin or LDH, hemoglobinemia, or the presence of bilirubin in the urine. In the same review, only 5 out of 14 (36\%) cases of anti-Jr\textsuperscript{a} examined showed MMA reactivity greater than 5\%, two of which had reactivity greater than 20\%. But the positive predictive value of the MMA result for hemolysis is not established specifically for anti-Jr\textsuperscript{a} because of the small number of cases and the lack of associated clinical information. Through a personal communication with these authors, we learned that clinical information was available on 4 of the 15 cases. Two of these cases had MMA results greater than 20\%. Of these, one experienced a transfusion reaction with the infusion of a third unit of Jr(a+) RBCs. This is the same case reported by Kwon et al.\textsuperscript{6} In the other case, flow cytometry showed normal RBC survival compared with the calculated expected RBC survival rate. Clinically the patient did not have signs of hemolysis. Clinical information on the other 11 patients is unavailable. In our case, despite the strong MMA reactivity both before and after the transfusion of a total of 15 units of incompatible RBCs, we found no clinical evidence of hemolysis and only laboratory evidence of very mild, well-tolerated, delayed hemolysis. The decrease in Hct was at least in part due to dilutional anemia and postoperative surgical blood loss.

It is likely that most transfusions of Jr(a+) RBCs will not result in significant acute or delayed hemolysis in the presence of anti-Jr\textsuperscript{a}. However, because anti-Jr\textsuperscript{a} can cause significant acute hemolysis at least in rare cases after repeated exposures to Jr(a+) RBCs\textsuperscript{6}, it is prudent to make a reasonable attempt to locate Jr(a–) RBC units when anti-Jr\textsuperscript{a} is identified, especially in those cases with strongly positive MMA results. If blood loss can be anticipated, as in the case of an elective surgery or delivery, autologous donations should be encouraged. It may also be necessary to look into family members and rare donor registries as potential sources of compatible units because of the extreme scarcity of the Jr(a–) phenotype in North America. It is important to weigh the availability of Jr(a–) RBCs against the cost and the infectious disease risks when importing such units from countries outside the United States or older units frozen before the availability of the most current infectious disease testing. In our opinion, the risks of acquiring an infectious disease appear to outweigh the risks of transfusing Jr(a+) units, and clinicians should be guided in this way. Additionally, clinicians should be advised of the available serologic results, which might include antibody titer, DAT reactivity, and MMA results. The limitations of these data and the lack of conclusive information in the current literature regarding the clinical significance of anti-Jr\textsuperscript{a} must be made clear as well. The clinical decision to transfuse Jr(a+) RBCs and the risks of hemolysis after transfusion of incompatible RBCs must also be evaluated against the risks of withholding transfusion. The recent guidelines of the UK National Blood Service\textsuperscript{17} recommended transfusing serologically least-incompatible RBCs to patients with anti-Jr\textsuperscript{a} and antigen-negative RBCs to patients with strong anti-Jr\textsuperscript{a} when transfusion is necessary. We feel this approach is reasonable given the rarity of Jr(a–) RBC units, the lack of data we have about anti-Jr\textsuperscript{a}, and the lack of laboratory assays that can reliably predict its clinical significance.
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Is there a relationship between anti-HPA-1a concentration and severity of neonatal alloimmune thrombocytopenia?

H. Bessos, M. Turner, and S. Urbaniak

There is uncertainty about the relationship between anti-HPA-1a levels and severity of neonatal alloimmune thrombocytopenia (NAIT). To investigate this relationship further, the concentration of anti-HPA-1a in HPA-1b homozygous women was determined, using a newly developed quantitative ELISA that uses purified anti-HPA-1a to obtain a standard curve. Seventy-eight samples collected from 22 HPA-1b homozygous pregnant women at various stages of pregnancy were tested. These included five women who had delivered babies with severe NAIT. A national HPA-1a antibody standard (NIBSC 93/710), designated as 1 arbitrary unit/mL (AU/mL), was used in each ELISA to calibrate the purified anti-HPA-1a, enabling the presentation of results as AU/mL. Moreover, selected samples were also assayed by PAK 12 and their reactivity compared with quantity of antibody. The use of the purified HPA-1a antibody yielded consistent sigmoid curves, enabling the measurement of HPA-1a antibody concentration in the test samples. The antibody concentration was significantly correlated with the antibody titer in the 78 samples studied (R = 0.54, p < 0.001). Furthermore, there was a significant correlation between PAK 12 and the quantitative ELISA in a selected number of cases, with or without NAIT (R = 0.71, n = 10; p < 0.02). On the other hand, there was no correlation of antibody concentration with NAIT incidence (R = –0.046). This study indicates that there is no relationship between anti-HPA-1a concentration and severity of NAIT when ELISA is used, although the correlation between ELISA and other methods, such as monoclonal antibody immobilization of platelet antigens (MAIPA) assay, remains to be determined. *Immunohematology* 2005;21:102–108.

Key Words: platelets, anti-HPA-1a, neonatal alloimmune thrombocytopenia (NAIT)

Neonatal alloimmune thrombocytopenia (NAIT) occurs when the pregnant woman and her fetus differ in their platelet glycoprotein (GP) polymorphisms and the woman develops an antibody to the fetal platelet antigens. In the Caucasian population, most severe cases of NAIT occur when the woman and fetus differ in their HPA-1 phenotype.1–3 About 10 percent of HPA-1b homozygous women who are pregnant with a fetus expressing HPA-1a antigens develop antibodies to the fetal platelets, which leads to NAIT in about 20 percent of these cases.1–3 This can lead to internal hemorrhage, of which the most serious form is intracranial hemorrhage (ICH), which can cause the death or lifelong morbidity of the baby.4 As NAIT is caused by the passage of IgG across the placenta, it is reasonable to assume that a relationship would exist between the isotype of anti-HPA-1a, its concentration, or both and the severity of the disease, as has been observed for HDN.5 However, to date, evidence of the relationship between HPA-1 antibody titer and the severity of NAIT has been inconclusive. Most studies have shown that no such relationship exists,2,6–12 while a few studies have shown a notable or significant correlation between the HPA-1a antibody isotype or concentration and the severity of NAIT.13–16 In a recent nationwide study in Scotland involving 26,506 pregnant women, 8.8 percent (28/327) of confirmed HPA-1b homozygous women developed HPA-1a antibody. Of those, eight delivered babies with NAIT, which in five of them was severe (platelet count < 50 × 10^9/L).17 Antibody titers during pregnancy were monitored by a direct ELISA,18 and no correlation between antibody titer and severity of NAIT was observed.

The previously mentioned discrepancy in the relationship between antibody levels and severity of NAIT could be due to the analytic methods used, because these are, in general, qualitative or semi-quantitative. For example, in ELISA methods, patient samples are incubated with purified GPIIb/IIa coated onto the well,10,18 while in other assays, patient samples are incubated with intact platelets.19,20 In the monoclonal antibody immobilization of platelet
antigens (MAIPA) assay, platelets are incubated with the patient’s antibodies before they are washed and incubated with monoclonal antibodies directed against various GPs. The GP, bound with the patient’s antibody and respective monoclonal antibody, is then extracted and tested further to determine the specificity of the patient’s antibody. Recently we developed an ELISA that enables quantitative measurement of antibody in patients’ serum. The aim of this study was to use this assay to confirm whether there is a relationship between the concentration of anti-HPA-1a and severity of NAIT in the cohort of pregnant women with anti-HPA-1a in our study. The correlation between the direct ELISA titer and the quantitative assay was also determined.

Materials and Methods

ELISA

Using immobilized HPA-1a, anti-HPA-1a was purified from serum containing a high titer (256) of this antibody; the purified anti-HPA-1a was used to generate a standard curve in the ELISA as previously described. The quantitative antibody ELISA consisted of the following steps, all of which were performed at room temperature. Seven serial doubling dilutions of the standard (purified) anti-HPA-1a and up to three serial dilutions of the test samples were incubated in HPA-1a–coated wells for 30 minutes. Controls consisting of the same constituents were incubated in uncoated wells. After washing, the wells were incubated with alkaline-phosphatase conjugated anti-human IgG for 30 minutes. The wells were again washed and incubated with substrate for 30 minutes. Stop solution was added and absorbances were obtained using an ELISA reader (MRX<sup>TC</sup>, Dynex Technologies, Chantilly, VA). Specific absorbance was obtained by subtracting the absorbance in uncoated wells (i.e., without antigen) from that in wells coated with antigen. Normal serum was not included in the test because a study of 94 normal antenatal samples had yielded a mean absorbance plus two SD of less than 0.1, indicating no cross-reactivity of normal samples with coated GPIIb/IIIa. All ELISA buffers and reagents were obtained from Axis-Shield plc, Dundee, Scotland. The purified anti-HPA-1a was used in the ELISA at a starting dilution of 1 in 4 (added as one part anti-HPA-1a and 3 parts buffer) in buffer containing 25% of normal pool plasma, with serial doubling dilutions to 1 in 256. This gave a sigmoid curve against the linear section of which test plasma samples could be measured (see Results section). To obtain universal results, the purified anti-HPA-1a was calibrated against a national minimum-potency anti-HPA-1a standard (NIBSC 93/710) designated as 1 arbitrary unit per mL (AU/mL).

Test samples

As previously indicated, 28 of 327 HPA-1b women who consented to our nationwide study were found to have anti-HPA-1a. Of these women, eight delivered babies with NAIT, five of whom had severe thrombocytopenia. At the end of the latter study, samples from 22 women were additionally tested for antibody, using the quantitative ELISA; this study included samples from all five women who had
delivered babies with severe NAIT. Eight consecutive ELISAs were performed to measure 78 samples collected from the 22 women at various stages of pregnancy. In addition, 10 selected samples (at delivery or postdelivery) were tested using PAK 12 (GTI, Waukesha, WI) to determine whether the latter correlated in any way with the ELISA. It is worth noting that PAK 12 also incorporates GPIIb/IIIa-coated wells, although the GP is attached to the wells via a monoclonal antibody and not directly as in our ELISA.

Statistics

The correlation between the titer or absorbance in the PAK 12 and the antibody concentration was calculated using the Pearson product moment correlation coefficient. The significance in the difference of antibody quantity between low and high titer samples was determined using the Wilcoxon t test.

Results

Consistency of the anti-HPA-1a standard

As before, consistent sigmoid curves were obtained by the purified anti-HPA-1a standard in the ELISA (data not shown). In each assay, the NIBSC 93/710 anti-HPA-1a (designated 1 AU/mL) was assayed at a 1 in 4 dilution and the amount of antibody in the purified anti-HPA-1a standard was calculated as shown in Figure 1. This yielded an average value of 25 AU/mL for the purified anti-HPA-1a. Such calibration of the purified anti-HPA-1a in each ELISA enabled measurement of the test samples in standard quantitative units (AU/mL).

Amount of antibody in the test samples

Seventy-eight samples obtained from various stages of pregnancy of 22 women with anti-HPA-1a were assayed. Thirty-four weakly reactive antibody samples of titers less than or equal to 2 gave a mean antibody quantity of 0.58 AU/mL (± 0.42 SD). In contrast, 16 strongly reactive antibody samples with titers between 32 and 256 gave a significantly higher mean antibody concentration of 4.27 (± 3.44) AU/mL, p < 0.0005. Overall, the antibody concentration was significantly correlated with the titer in the 78 samples studied (R = 0.54, p < 0.001) (Fig. 2). Furthermore, there was a significant correlation between the results of PAK 12 and the quantitative ELISA (Fig. 3). However, no relationship was found between antibody concentration and severity of NAIT (R = -0.046).

Indeed, as shown in Figure 4, antibody concentration barely rose above 1 AU/mL during pregnancy in three of the five severe NAIT cases (charts B, D, and E; respective platelet counts are shown in the figure legend); in contrast, in two cases of normal delivery platelet counts, appreciably higher concentrations of 4 to 10 AU/mL were observed in the third trimester (charts H and I). Antibody concentrations of between 1 and 6 AU/mL were observed during pregnancy in the remaining two severe NAIT cases (charts A and C). The selected cases (10/22) in Figure 4 highlight the variability of the relationship between antibody
Fig. 4. Antibody concentration in 10 representative women with anti-HPA-1a during pregnancy.

D = delivery, PN = postnatal. All platelet counts at delivery (× 10^9/L).

A to E: Severe NAIT. Respective platelet counts: 21, 13, 14, 8, and 42. F: Mild NAIT. Platelet count: 87. G to J: No NAIT. The concentration of antibody during pregnancy did not appear to be indicative of the severity of NAIT.
concentration and severity of NAIT. All five women who gave birth to babies with severe NAIT were HLA-DR3*01 positive (data not shown). The thrombocytopenia resolved without treatment in one of the babies, while those of the other four women received one or two transfusions with HPA-1b homozygous platelets before their platelet counts returned to normal.¹⁷

Discussion

In this study, the relationship between anti-HPA-1a concentration and severity of NAIT was assessed using a newly developed quantitative ELISA. During our nationwide Scottish study we did not find a correlation between antibody titer and severity of NAIT. Our aim, therefore, was to determine whether such a correlation would be observed upon accurate quantitation of antibody in samples obtained from the same cohort. Seventy-eight samples collected from 22 antibody-positive women during pregnancy were tested. A significant correlation was observed between antibody titer and concentration (i.e., between the direct ELISA and the quantitative ELISA, respectively). Based on this correlation, it was not surprising that no relationship was observed in the current study between antibody concentration and severity of NAIT, since the latter was not related to antibody titer either.

Our findings are in keeping with those of many other studies where investigators have failed to find such a relationship.⁶–¹² In a study by Kaplan et al.,¹⁸ one in five mothers who delivered thrombocytopenic babies did not have detectable antibodies. A similar observation was made by Bussel et al. in a study of seven pregnant women who had previously delivered infants with severe NAIT. Two of these seven women, whose fetuses developed thrombocytopenia during the early phase of their subsequent pregnancy, had no detectable antibody. However, based on their previous history, IVIG was infused into these women in an attempt to raise the platelet count of the fetuses before delivery. Using an ELISA similar to ours, Proulx et al. analyzed the sera from 36 mothers who gave birth to thrombocytopenic babies. Their results indicated that neither the titer nor the isotype of the antibody could predict the severity of NAIT. In contrast, Mawas et al. found a significant increase of anti-HPA-1a of the IgG3 immunoglobulin class in women who delivered severely thrombocytopenic babies, although the levels of IgG1, IgG2, and IgG4 (and overall IgG levels) remained the same between the two groups. However, the method they used was a modified MAIPA assay using HPA-1a homozygous platelets and not ELISA plates coated with HPA-1a GPIIb/IIIa GP. Likewise, in two other recent studies where MAIPA assay was used, a relationship was found between antibody titer and quantification and severity of NAIT.¹⁴–¹⁵ In the study in East Anglia, England, by Williamson et al.,¹⁴ a significant correlation between severity of NAIT and a third trimester anti-HPA-1a titer of less than 32 was found. Moreover, in a smaller study by Jaegtvik et al. in Norway, an obvious relationship between antibody level at the time of delivery and the severity of NAIT was observed. Nevertheless, two women with barely detectable antibodies delivered babies with severe NAIT. In addition, one woman with high antibody levels delivered a baby with mild NAIT (cord platelet count of around 125 × 10⁹/L), but this was attributed to the protective effect of anti-D that was also present in her serum. In a recent study by Maslanka et al.,¹⁶ where 144 of 8013 pregnant women were found to be HPA-1b homozygous, 12 of 122 of the HPA-1b women produced anti-HPA-1a (as determined by MAIPA assay); two of the babies had severe antenatal thrombocytopenia and two had mild NAIT. Due to the small number of cases, no definitive conclusion could be drawn regarding the relationship of antibody titer to severity of disease. However, the four thrombocytopenic cases occurred in mothers who had antibody titers of 4 or higher, while no thrombocytopenia was observed in those mothers who had weakly reactive anti-HPA-1a detectable only after delivery.

Although antibodies to other human platelet glycoprotein polymorphisms, such as HPA-5b and HPA-4b, may have mechanisms of action that differ from those of anti-HPA-1a, it is worth noting that discrepancies have also been noted with such antibodies in relation to severity of NAIT.²⁴–²⁶ While a study by Kurz et al. did not find HPA-5b antibody titer to be a predictor of disease, a study by Ohto et al. found significant association between postnatal platelet concentration (Day 3) and anti-HPA-5b titer in the third trimester of greater than or equal to 64. In addition, no relationship between anti-HPA-4b and severity of NAIT was observed in a large Japanese study. (Antibodies in the latter two studies were determined by means of a mixed passive hemagglutination assay.)

It is clear that there are no consistent results illuminating the relationship of anti-HPA-1a concentration and severity of NAIT. The discrepancy between the various studies could be due to the use of different assays. It appears that all those who have used ELISA
incorporating GP-coated wells have failed to find a correlation between anti-HPA-1a levels and severity of NAIT, whereas all those who have found such a relationship have used a MAIPA assay. However, this is not to say that the use of a MAIPA assay always yields such a correlation. In this study we calibrated the purified anti-HPA-1a standard in each assay against NIBSC 93/710, designated as 1 AU/mL. This enabled the quantitation of anti-HPA-1a in all of our samples as AU/mL. Three of five women who had delivered babies with severe NAIT exhibited consistently low concentrations of anti-HPA-1a throughout their pregnancies, barely exceeding 1 AU/mL. Although the remaining two women in this group exhibited higher antibody concentrations, the amounts interestingly declined in the second trimester, rising again in the third trimester and postdelivery. In contrast, two women with high, albeit fluctuating, antibody concentrations from 4 to 10 AU/mL during pregnancy delivered babies with normal platelet counts. Our results, therefore, indicate that the antibody quantification during pregnancy is not a reliable predictor of severity of NAIT. However, an inverse relationship between antibody levels and platelet counts in utero cannot be ruled out, nor can the effect of variation of maternal blood volume in our cohort of pregnant women; it is conceivable that some of those with low antibody levels could have had larger blood volumes than those with high antibody levels.

Based on the apparent discrepancy between ELISA-based test systems and MAIPA assays, it would be useful to carry out a comparison between MAIPA assay and GPIIb/IIIa-coated quantitative ELISAs using the same samples. In the meantime, in the absence of a consensus on the role of anti-HPA-1a concentration in NAIT, the clinical outcome (antenatal ICH), the degree of thrombocytopenia (platelet count < 20 × 10⁹/L) in previously affected pregnancies, or both appear to be the predictors of the likely severity of fetomaternal alloimmune thrombocytopenia, though only in subsequently affected pregnancies.²⁷

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References


The complement system plays a crucial role in fighting infections and is an important link between the innate and adaptive immune responses. However, inappropriate complement activation can cause tissue damage, and it underlies the pathology of many diseases. In the transfusion medicine setting, complement sensitization of RBCs can lead to both intravascular and extravascular destruction. Moreover, complement deficiencies are associated with autoimmune disorders, including autoimmune hemolytic anemia (AIHA). Complement receptor 1 (CR1) is a large single-pass glycoprotein that is expressed on a variety of cell types in blood, including RBCs and immune cells. Among its multiple functions is its ability to inhibit complement activation. Furthermore, gene knockout studies in mice implicate a role for CR1 (along with the alternatively spliced gene product CR2) in prevention of autoimmunity. This review discusses the possibility that the CR1 protein may be manipulated to prevent and treat AIHA. In addition, it will be shown in an in vivo mouse model of transfusion reaction that recombinant soluble forms of CR1 can reduce complement-mediated RBC destruction, thereby prolonging survival of transfused RBCs. It is proposed that CR1-based therapeutics have potential for effective and safe prophylactic short-term use and for treatment of hemolytic transfusion reactions. *Immunohematology* 2005;21:109–118.

**Key Words:** complement, complement receptor 1, sCR1, inhibitors, LHR, immune hemolysis, autoimmune hemolytic anemia, transfusion medicine, regulatory T cells, CD4⁺CD25⁺

**The Complement System and Red Cell Destruction**

The complement system is an important part of the innate immune system for fighting infections and foreign molecules before an adaptive response has developed.¹ It is also an important regulator of B-cell, and possibly T-cell, immunity.² ³ However, it can also cause cellular and tissue damage when activated inappropriately, contributing to many clinical conditions, including reperfusion injury (following surgery, ischemic disease, and organ transplantation), organ rejection, acute inflammatory injury to the lungs, and autoimmune diseases.¹ For the system to exert its biological activities, it has to be activated. Activation occurs in a sequence that involves proteolytic cleavage of the complement components, resulting in the release of active biological mediators and the assembly of active enzyme molecules that result in cleavage of the next downstream complement component.¹ Depending on the nature of the activators, three complement activation pathways have been described: the antibody-dependent classical pathway and the antibody-independent alternative and lectin pathways (Fig. 1).¹ Common to all three pathways are two critical steps: the assembly of the C3 convertase enzymes and the activation of C5 convertases. Specifically, the C3 convertases cleave C3 into C3a, a potent anaphylatoxin, which acts as a neutrophil chemotaxin and activator,⁴ and C3b, which covalently attaches to nearby targets, where it directs immune clearance and antigen selection.⁵ In addition, the C5 convertases cause the release of another potent anaphylatoxin, C5a,⁴ and lead to the formation of the pore-like membrane attack complex (C5b-9), which inserts into cell membranes, causing lysis or sublytic damage to the target cell (Fig. 1).⁵

In the transfusion medicine setting, complement-mediated RBC destruction plays a critical role, being involved in both intravascular and extravascular hemolysis.⁶ Generally, in the presence of a potent, complement-binding antibody and large numbers of closely situated RBC antigens, complement activation can proceed to completion, resulting in intravascular hemolysis, which can be fatal.⁷ ⁸ However, the majority of blood group antibodies (including both alloantibodies and autoantibodies) that can fix complement activate complement up to the C3 stage but do not go on to act as hemolysins.⁹ Although antibody-coated RBCs can also be destroyed extravascularly without complement activation, RBC removal by tissue
macrophages in the spleen and liver is enhanced considerably when C3 is present on RBCs in addition to IgG. Indeed, as many as 50 percent of patients with autoimmune hemolytic anemia (AIHA) have both IgG and complement on their RBCs.

**Complement Regulatory Proteins and CR1**

The complement system can be divided into three separate pathways (classical, alternative, and lectin), depending on the type of activator. The system consists of nearly 30 different serum and membrane proteins which, after activation, interact in a highly regulated enzymatic cascade to generate reaction products that mediate inflammation and host protection. Because of the direct and indirect powerful cytolytic activity of complement, there exists a family of structurally and functionally related proteins, known as regulators of complement activation (RCA), that prevent potential host cell damage from complement activation (Fig. 1). CR1, also known as CD35, is the most versatile of the RCA family because it exhibits decay-accelerating and cofactor properties that can inactivate the two critical enzymes of the complement activation pathways (Fig. 1 and Fig. 2).

Specifically, by binding to C4b or C3b, CR1 can displace the catalytic subunits (decay-accelerating activity [Fig. 2]) of the convertases. In addition, by acting as a cofactor for plasma protease factor I, CR1 is responsible for the degradation of C4b and C3b (Fig. 2), and thus complete inactivation of the convertase. CR1 has also been shown to function as a receptor for C1q, the first component of the classical pathway as well as the mannan-binding lectin (MBL) of the lectin pathway.

**CR1 Expression Pattern and Functions**

CR1 is expressed on a number of cell types, mostly in the blood, and at low levels in soluble form in the plasma. Of the C4 and C3 regulatory proteins, CR1 has the widest range of activities and functions, including immune complex clearance, regulation of complement activation, phagocytosis, and antibody response, as well as deletion of autoreactive B and maybe T cells. Through its ability to bind C3b and C4b, erythrocyte CR1 transports immune complexes to the spleen and liver for their removal. CR1 expressed on macrophages and neutrophils mediates adherence and phagocytosis, that expressed on B cells modulates the threshold for B-cell activation, and that on follicular dendritic cells is thought to improve the immune response to antigen. Through its ability to bind C3b and C4b, erythrocyte CR1 transports immune complexes to the spleen and liver for their removal. Moreover, CR1 is expressed on 10 to 15 percent of human T lymphocytes, although its function on such cells is not clear. The expression pattern of murine CR1 is for the most part similar to that of human CR1 except that mouse RBCs do not express CR1. To date, no individuals completely
lacking the CR1 protein have been identified. However, RBCs that express low copy numbers of CR1 and its associated Knops blood group antigens, known as the Helgeson phenotype, have been described and appear to be associated with protection against severe malaria.\textsuperscript{42}

**CR1 Structure**

In humans, four allotypes of CR1, varying in size, are known.\textsuperscript{43} The predicted amino acid sequence of the common allotype (CR1*1) is 2039 residues (Fig 3).\textsuperscript{18,44,45} The extracellular 1930-residue-long domain of CR1, with 25 potential N-glycosylation sites, can be divided into 30 short consensus repeats (SCRs), each of 59 to 72 amino acids (aa) with sequence homology between SCRs ranging from 60 to 90 percent.\textsuperscript{45} Homologous SCRs with four conserved cysteine residues are also found in other RCA members.\textsuperscript{12} The first 28 SCRs of CR1 are further arranged into four longer regions of similarity, termed long homologous repeats (LHRs A through D), consisting of seven SCRs each\textsuperscript{18,44} (Fig. 3). In mice, CR1 is expressed along with the alternatively spliced gene product CR2.\textsuperscript{46–48} Murine CR1 consists of 21 SCRs, with 15 SCRs identical to murine CR2 and 6 unique SCRs at the amino terminus.\textsuperscript{46–48}

\textbf{Fig. 3.} Schematic representation of the common isotype of CR1. The 30 SCRs are represented by blocks. Groups of seven SCRs are further subdivided into four LHRs. The ligand binding sites for C3b and C4b are shaded. The preferred ligand is in bold; the alternative ligand is in parentheses. The positions of C1q/MBL binding sites have not yet been defined.

**CR1-Based Therapeutics**

The complement regulatory function of CR1 has been exploited for development of a potent anti-complement agent. Specifically, a recombinant soluble form of CR1 (sCR1), by binding C3b and C4b and inactivating the convertases, has successfully inhibited the complement activation cascade and prevented complement-mediated tissue injury in several animal models.\textsuperscript{49,50} More importantly, sCR1 has been in human clinical trials for the treatment of acute respiratory distress syndrome and to reduce tissue damage in myocardial infarction and lung transplantation,\textsuperscript{51–53} with possible favorable outcomes.\textsuperscript{52} Despite the role of CR1 as a global inhibitor of complement activation, the antibacterial defenses of the patients undergoing treatment have not, thus far, been compromised, underscoring the usefulness of sCR1 as a therapeutic agent.\textsuperscript{53} It is important to note that there are currently no anticomplement therapeutics in the clinic. Indeed, sCR1 represents the best in vivo characterized anticomplement agent to date.

Our studies are the first to demonstrate a potential for sCR1 for inhibiting complement-mediated RBC destruction following transfusion immunization events.\textsuperscript{53,54} Furthermore, through structure-function analysis we have identified a 254-aa domain at the N-terminus of CR1, consisting of four SCRs and one-eighth of sCR1, that has antihemolytic activity in vivo. Previous in vitro studies by us and others demonstrated that the N-terminal domain of CR1 has barely any cofactor activity, but has decay-accelerating activity for the C3 convertases and inhibits the classical activation pathway.\textsuperscript{54,56–61} Moreover, we and others have shown that the four N-terminal SCRs preferentially bind C4b.\textsuperscript{45,56,59,62–64} Based on these observations, we believe that fine mapping of the C4b binding and the decay-accelerating activity (C4b.2a) for the classical activation pathway in the 254-aa domain will lead to future design of nonimmunogenic small molecule inhibitors to down-regulate complement-mediated RBC destruction. It is important to note that chronic treatment with complement inhibitors is likely to undermine the body’s ability to fight infections\textsuperscript{65} and may lead to development of autoimmune diseases (discussed in a later section). The application for these inhibitors is thus for short-term prophylactic use before transfusion of not fully matched blood in emergency situations and as a therapeutic option in select patients with complement-mediated immune hemolysis to ameliorate the life-threatening complications.

**Complement and AIHA**

AIHA is an autoimmune disease caused by autoantibodies against RBC self-antigens, causing
shortened RBC survival. The underlying mechanism for breakdown of immunologic tolerance is not well established. Several lines of evidence support the role of complement in the maintenance of tolerance to self-antigens: (1) Hereditary deficiencies of complement proteins of the classical pathway have been associated with autoimmune diseases. For example, 90 percent of C1q-deficient patients develop autoimmune disease, and C1q knockout mice have a high incidence of developing autoimmune disease. (2) Altered levels of expression of CR1 have been observed in patients with AIHA as well as other autoimmune diseases, such as systemic lupus erythematosus, rheumatoid arthritis, Sjogren's syndrome, and some diabetic patients. In a mouse model of severe lupus-like disease (MRL/lpr), lower levels of CR1/CR2 receptors have been found on B cells before the development of disease manifestations, suggesting that altered complement receptor expression may contribute to initiation or progression of autoimmune disease. Gene knockout studies of mice lacking CR1 (along with the alternatively spliced gene product CR2) indicate that CR1 and CR2 control the activation thresholds of B cells to self-antigens, although the exact molecular mechanism is not clearly understood. Because CR1/CR2 knockout mice lack both CR1 and CR2, the specific contribution of each receptor cannot be dissected. However, mice deficient in C4, but not C3, have a phenotype similar to CR1/CR2-deficient mice in studies demonstrating their role in immune tolerance, strongly suggesting that the primary effect in these mice is mediated by CR1. (3) C3b was recently shown to induce the development of T-regulatory cells, known to be important for maintenance of peripheral tolerance (discussed in a later section). Specifically, it was shown that membrane cofactor protein (MCP, CD46), a natural complement regulatory protein whose ligands are C3b and C4b, can act as a coreceptor for inducing the development of IL-10-secreting CD4+CD25+ regulatory T cells, which are responsible for active suppression of autoreactive T cells.

T-Regulatory Cells
T-regulatory cells (Tregs) are a subset of T cells that function to control immune responses, including those directed against self-antigens. Different populations of Tregs have been described, including thymically derived naturally occurring cells and those that are induced in the periphery through exposure to antigen. Naturally occurring Tregs constitute about 1 to 2 percent of peripheral blood mononuclear cells, or about 5 to 10 percent of the CD4+ T cells, and are characterized by coexpression of CD25 (α subunit of IL-2 receptor) and the transcriptional repressor FoxP3. Their role in maintenance of self-tolerance and their ability to suppress a number of autoimmune diseases has attracted a great deal of attention and opened the possibility of developing novel immunotherapeutic strategies for suppression of autoimmunity. Nevertheless, many questions remain to be answered about the characteristics and biology of Tregs. For example, it is not known whether the suppressive activity of the CD4+CD25+ Tregs can be subdivided to smaller subpopulations or whether smaller individual CD4+CD25+ Tregs have different degrees of suppressive activity. Supporting this possibility, CD103+ T cells within CD4+CD25+ Tregs were shown to have more suppressive activity than CD103+CD4+CD25+ Tregs and CD62L+ expressing CD4+CD25+ Tregs appear more potent in preventing certain autoimmune diseases in mice. Preliminary studies in my laboratory indicate that CR1 is indeed expressed on a subpopulation of CD4+CD25+ Tregs. The molecular basis of the mechanism of Treg cell-mediated suppression is not fully known, although the consensus is that they can expand and augment their suppressive activity when stimulated. In vitro naturally occurring CD4+CD25+ mediate suppression of cocultured CD25- T cells by cell-cell interactions, not by cytokines. Activation has been shown to occur through TCR-specific signals. Interestingly, non-TCR-specific stimuli such as bacterial products through Toll-like receptor 4 have also been shown to activate CD4+CD25+ Tregs. Given that innate immune responses such as Toll-like receptors can stimulate Tregs, it is conceivable, although as yet untested, that complement activation products, through interactions with complement receptors such as CR1, may also be involved in augmenting or attenuating the activation state of Tregs, with consequences for their suppressive activity. In support of this possibility, C3b-CD46 interactions were recently shown to activate a subset of Tregs, namely the inducible Tregs. Preliminary studies from my laboratory indicate that CR1 can also mediate the suppressive activity of CD4+CD25+ Tregs, although the exact mechanism of suppression is still under investigation.
Possible Role of Complement/CR1 in Treg Induction

We speculate that after certain infections, complement is activated and foreign antigens that are opsonized with complement components C3b and C4b, which are known to be the ligands for CR1 and CD46, may induce regulatory T cells. Thus, the initial complement activation will help clear the pathogen by direct lysis, inflammatory response, phagocytosis of complement-sensitized infectious agent, or a combination of these (Fig. 1). In our hypothetical model, complement activation through induction of Tregs will also result in suppression of certain self-reactive lymphocytes as well as effector T cells that would normally cause infection-induced immunopathology (Fig. 4). Given that complement is so tightly regulated, it is conceivable that many factors, including the nature and strength of the activators (pathogens), will determine the balance among complement activation, Treg induction, and T-cell suppression. Our model obviously does not preclude the role of other mediators besides complement activation products for Treg activity, which may explain why certain infections persist or become chronic and are correlated with lower incidence of autoimmunity. Nevertheless, complement deficiencies, which we propose can cause decreased Treg activity, are associated with autoimmune disease. AIHA appears to be a secondary complication in patients with a range of diseases, including those with viral or mycoplasmal infections, and the presence of cross-reactive foreign antigens may be the underlying mechanism for breakdown of tolerance in these patients.

It is interesting to note that Rh antibodies do not fix complement on RBCs. In warm-type AIHA, many of the autoantibodies are directed against the Rh complex. It may be that antigens such as Rh that are not initially opsonized with complement components may not be subject to immune tolerance. Thus, upon stimulation with cross-reactive foreign antigens, autoreactive T cells specific to Rh antigens are preferentially expanded, resulting in AIHA. Future studies are needed to further explore the potential contribution of CR1 and its ligands in the development of AIHA.

Acknowledgments

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Autoimmune hemolytic anemia and a further example of autoanti-Kp<sub>b</sub>

E. Lee, G. Burgess, and N. Win

A 65-year-old Caucasian man with myelodysplasia was admitted with autoimmune hemolytic anemia and a Hb of 5.6 g/dL. The patient’s serum contained anti-K; the DAT on the patient’s RBCs reacted 3+ with anti-IgG and 3+ with anti-C3d. K- RBC units were transfused, but there was no sustained increase in Hb level. The samples were referred to the reference laboratory of the National Blood Service. The DAT results remained the same, with anti-K detected only in the serum. An eluate prepared from the patient’s DAT-positive RBCs revealed anti-K<sub>b</sub> specificity. This study reports an unusual case of autoanti-Kp<sub>b</sub>, which is different from previously published cases in that no free anti-Kp<sub>b</sub> was detectable in the serum. Immunohematology 2005;21:119–121.

Key Words: autoimmune hemolytic anemia, anti-Kp<sub>b</sub>

Autoantibodies found in patients with warm autoimmune hemolytic anemia (WAIHA) often show a RBC specificity related to the Rh system. The first example of this type of autoantibody was described by Race and Sanger. Although antibodies to other high-prevalence antigens have been reported, such as autoanti-AnWj, -Co<sub>b</sub>, -En<sup>a</sup>, -Ge, -Hr<sup>b</sup>, -Kp<sub>b</sub>, -K13, -Kx, -LW, -N, -Rh34, -Sc1, -Sc3, -U, -Vel, and -Wr<sup>b</sup>, they are rare. We report an unusual case of autoanti-Kp<sub>b</sub>, which is different from previously published cases in that no free anti-Kp<sub>b</sub> was detectable in the patient’s serum. Kp(b−) RBCs were issued for further transfusion support. Transfusion of presumably Kp(b+) RBCs did not result in a sustained increase in Hb, therefore Kp(b−) RBCs were issued for further transfusion support. Subsequent transfusions were uneventful and achieved prolonged Hb increment.

Case Report

A sample from a 65-year-old Caucasian man with myelodysplasia, on regular transfusion support, was tested to be Group O, R<sub>R1</sub>, R<sub>R2</sub> with anti-K in the serum. Later the patient had colitis and developed acute autoimmune hemolytic anemia (AIHA) with a positive DAT. The patient was transfused with K- RBCs but there was no sustained response in Hb level. The samples were submitted to the Reference Laboratory of the National Blood Service (NBS), North London, for investigation.

Materials and Methods

Antigen typing for ABO, Rh, and K were performed by standard tube technique. Anti-A and anti-B reagents were from Diagnostic Scotland, Edinburgh, Scotland; anti-D reagents (RUM1 and BS226) were from NBS Reagents, Cambridge, United Kingdom; monoclonal anti-Rh (anti-C, -E, -c, and -e, ), and anti-K were from BIOSCOT (now Serologicals), Ltd., Livingston, Scotland. Antibody identification was performed by standard tube LISS-IAT (LISS from Inverclyde Biologicals, Bellshill, Scotland). The patient’s serum was tested with an in-house panel of RBCs to determine antibody specificities. The manual polybrene technique was also used to determine additional antibody specificities. The 0.05% working solution of polybrene, the resuspending solution, and low-ionic-strength medium were prepared in house according to Methods in Immunohematology. The DAT was performed with polyspecific AHG and monospecific anti-IgG and anti-C3d reagents (NBS Reagents). An eluate was prepared from the patient’s RBCs using chloroform following the protocol in Methods in Immunohematology and was tested with in-house selected-RBC panels and with RBCs from the frozen inventory for antibody identification. Chloroquine diphosphate (CDP, 200 mg/mL, pH 5.0 ± 0.1) was prepared by NBS Reagents. CDP treatment consisted of adding four volumes of CDP to one volume of washed packed RBCs, mixing, and incubating at 20°C for a maximum of 2 hours. A DAT using anti-IgG was performed on the CDP-treated RBCs every 30 minutes during this incubation to ensure the effectiveness of the CDP treatment.
Results

A posttransfusion sample from the patient showed the RBCs to be Group O, R1 R2 and to have a positive DAT, reacting 3+ with both anti-IgG and anti-C3d. Tests by routine LISS-IAT, saline, and manual polybrene techniques revealed anti-K in the patient’s serum. An eluate was prepared using a chloroform method and autoanti-Kp(b) was detected, reacting 3+. After CDP treatment of the patient’s RBCs, they were typed as Kp(b+) (Table 1). The patient was transfused with two units of K-, Kp(b–) RBCs. Three additional units of K-, Kp(b–) RBCs were transfused two days later. All of the units were frozen-recovered, deglycerolized RBCs from the same donor. The transfusions were well tolerated and the patient recovered from this hemolytic episode.

One month later, a DAT performed on the patient’s RBCs was still positive (anti-IgG 3+, anti-C3d weak positive), and anti-K was detected by LISS-IAT in the patient’s serum in addition to a weak autoantibody detectable by manual polybrene technique only. Autoanti-Kp(b) was not apparent in the serum or eluate. Although all of the RBCs transfused to the patient were K–, anti-K was eluted from the patient’s RBCs, probably due to the Matuhasi-Ogata phenomenon, in which alloantibodies are nonspecifically bound to the RBC along with autoantibody (Table 1).

Discussion

An autoantibody with Kp(b) specificity has been reported in AIHA and appears to be rare. In all previously reported cases of AIHA with autoanti-Kp(b) specificity, free autoantibody was always detectable in the serum. In the case we describe, no free autoanti-Kp(b) was detected, distinguishing it from the other published cases. The identification of autoanti-Kp(b) in this case was fortuitous, as the routine panel of reagent RBCs used at the time had one example of Kp(b–) RBCs, which helped to define the autoantibody specificity.

Most autoantibody specificities are Rh-related, such as anti-nl, anti-pdl, and anti-dl, and it is reported that up to 50 percent of antibody specificities in WAIHA are in these groups. Usually it is not necessary to honor the specificity of the autoantibody when selecting RBCs for transfusion. The use of a high-prevalence antigen-negative panel is often not indicated and the ability to resolve these specificities is limited by the availability of suitably typed reagent RBCs, such as Rhnull and Rh deletion. Extensive investigations are only performed in selected rare circumstances. In our institution, Rh- and K-matched RBCs are selected and issued for transfusion in cases of AIHA.

The patient in this case had pre-existing myelodysplasia, developing colitis, and infection. Although disease-associated transient suppression of Kell antigens has been reported, no suppression of the Kp(b) antigen was observed in this case. A positive result was obtained in the Kp(b) typing after CDP treatment of the patient’s RBCs. This finding may, however, be due to the timing of the test sample.

There are a few case reports of autoantibody with Kp(b) specificity described in the literature. Marsh et al. reported a case of AIHA, due to anti-Kp(b), in a patient who had a severe hemolytic transfusion reaction after receiving Kp(b+) RBCs. As previous transfusions with K– RBCs had not resulted in a sustained Hb increment, Kp(b–) RBCs were selected; transfusion was uneventful and an excellent hematologic response was achieved.

Win et al. also reported a rare case of AIHA in a 12-week-old infant. Autoanti-Kp(b) was identified in the infant’s serum. Initially the infant was transfused with Kp(b+) RBCs and had recurrent hemolytic transfusion reactions. Pronounced jaundice was noted and there was no sustained increase in Hb level after transfusion. The reference laboratory detected autoanti-Kp(b) in the serum and transfusion with Kp(b–) RBCs gave an excellent result with no further reactions.

Table 1. Summary of laboratory findings

<table>
<thead>
<tr>
<th>Date</th>
<th>Hb (g/dL)</th>
<th>Antibody (serum)</th>
<th>Antibody (eluate)</th>
<th>DAT</th>
<th>Phenotype of patient</th>
<th>RBC units provided</th>
</tr>
</thead>
<tbody>
<tr>
<td>5/15/98</td>
<td>NA</td>
<td>Anti-K</td>
<td>NT</td>
<td>Anti-IgG</td>
<td>NT</td>
<td>K–</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Anti-C3d</td>
<td>NT</td>
<td>2 units K–</td>
</tr>
<tr>
<td>6/4/98</td>
<td>5.6</td>
<td>Anti-K (5+)†</td>
<td>Anti-Kp(b) (3+)</td>
<td>3+</td>
<td>K–, Kp(b+)</td>
<td>2 units R,r, K–, Kp(b–)</td>
</tr>
<tr>
<td>6/11/98</td>
<td>7.0</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>–</td>
<td>3 units R,r, K–, Kp(b–)</td>
</tr>
<tr>
<td>7/10/98</td>
<td>NA</td>
<td>Anti-K (5+)†</td>
<td>Anti-K (2+)†</td>
<td>3+</td>
<td>(+)</td>
<td>None</td>
</tr>
</tbody>
</table>

*NA = not available, NT = not tested
†Refer to Table 2
‡LIP = manual polybrene technique
§Anti-K probably due to Matuhasi-Ogata phenomenon
The use of Rh- and K-matched RBCs for AIHA patients is standard practice in our organization and only rarely are RBCs that are negative for antigens against which autoantibodies have been detected selected for transfusion. In the case we describe here, because of the unusual nature of the autoantibody, and because of the lack of a response to the transfusion of Kp(b+) RBCs, we selected frozen-recovered, deglycerolized Kp(a+b–) RBCs for transfusion during the hemolytic episodes.

AIHA with autoanti-Kp\(^b\) specificity is rare and our case supports previous findings that Kp(b–) RBCs should be selected for patients with Kp\(^b\) autoantibodies for transfusion support.

**References**


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The incidence of red cell alloantibodies underlying panreactive warm autoantibodies

M. Maley, D.G. Bruce, R.G. Babb, A.W. Wells, and M. Williams

A recognized hazard of administering blood transfusions to patients with panreactive warm autoantibodies is that alloantibodies may be masked. Studies have shown the incidence of underlying alloantibodies to be 30 to 40 percent. Adsorption procedures can be used to remove autoantibodies and allow detection and identification of underlying alloantibodies. This study contains data from 126 patients referred to the Red Cell Immunohaematology laboratory at the National Blood Service, Newcastle upon Tyne, United Kingdom. These patients were from the northeast of England, a population for which data have not previously been reported. Samples identified as containing panreactive warm autoantibodies were subjected to adsorption procedures (95 by alloadsorption and 31 by autoadsorption). Absorbed sera were then tested to identify underlying alloantibodies. Of 126 samples, 39 (31%) contained a total of 61 RBC alloantibodies; 15 (12%) contained 2 or more antibody specificities; and 14 (11%) contained alloantibodies not found within the Rh or Kell blood group systems. Antibodies identified included the following specificities: E (19), D (9), c (7), C (6), S (5), Fy' (3), Jk' (2), Jk'' (2), K (2), Kp' (2), Fy' (2), Cw, N, and f (ce). This study reinforces the value of adsorption studies, whether using autologous or allogeneic RBCs, when panreactive warm autoantibodies are present. In addition, this study confirms that it is not appropriate in these cases simply to issue blood which is “least incompatible” or Rh phenotype- and K antigen-matched.

Materials and Methods

Our alloadsorption method uses at least two sets of strongly papain-treated donor RBCs selected to have complementary expression of important RBC antigens. It is our usual practice to use Group O, rr, K– and Group O, R, R, K– RBCs, one RBC sample being Jk(a–b+) and the other being Jk(a+b–). Reagent RBCs for adsorption were obtained from the UK NBS central reagents unit. Autoadsorption uses the patient’s papain-treated RBCs. Alloadsorption was performed on more cases as there was a limited supply of patients’ RBCs to use to perform autoadsorption, due to severe anemia or low sample volumes. Serial adsorptions were performed by incubating 1 mL of the patient’s plasma with 1 mL of the papain-treated packed RBCs at 37°C; the absorbed plasma was then retrieved by centrifugation. A maximum of 4 aliquots of allogeneic RBCs, or typically 2 aliquots of autologous RBCs, were used for this process. The number of autoadsorption procedures was dependent on the volume of RBCs available from the patient sample. Absorbed plasma was then investigated to detect and identify any underlying alloantibodies, using reagent RBCs suspended in low-ionic-strength red cell preservative solution (RCPS; Inverclyde Biologicals, UK) by tube IAT. If possible, phenotype studies were used to confirm that the identified antibodies were alloantibodies.
**Results**

Samples from 130 individual patients were referred for investigation of panreactive autoantibodies by autoadsorption or alloadsorption procedures. Data from four samples in which we failed to successfully remove the autoantibody were omitted from this study. Alloadsorption procedures were performed on 95 samples and autoadsorption procedures on 31 samples. Thirty-nine of 126 samples (31%) contained a total of 61 RBC alloantibodies (Table 1); 87 samples (69%) contained no underlying alloantibodies. Fifteen samples (12%) contained two or more specificities (Table 2). Fourteen samples (11%) contained alloantibodies with specificities for antigens outside the Rh or Kell blood group systems.

Of the 61 alloantibodies identified, all but 4 (anti-Kp\(^a\) [2], anti-N [1], and anti-C\(^w\) [1]) would require the selection of antigen-negative RBCs for crossmatching in the UK.\(^6\) Of 95 alloadsorbed samples, 33 (35%) contained alloantibodies, whereas 6 of 31 (19%) autoadsorbed samples contained alloantibodies.

All but six antibodies (anti-Fy\(^a\) [3], anti-Kp\(^a\) [2], and anti-Fy\(^b\) [1]) were confirmed as alloantibodies, using phenotyping studies. Phenotyping studies were not undertaken for these six patients as their RBC samples had strongly positive DATs. With only typing reagents requiring the use of AHG available and without a local procedure for removing autoantibody for typing, test results would be unreliable. These six antibodies were therefore assumed to be probable alloantibodies. Autoantibodies of these specificities are rarely reported.

**Discussion**

**Limitations and advantages of the adsorption procedures**

1. **Alloadsorption**

Alloadsorption procedures were preferentially performed in the cases described, but a number of factors must be borne in mind when interpreting results. Alloadsorption procedures require a minimum of two individual RBC samples with a complementary antigenic profile at key antigens, in our study, Rh, K, Jk\(^a\), and Jk\(^b\) antigens, and at least 2 mL of available patient plasma or serum. Papain treatment of the adsorption RBCs removes enzyme-labile antigens, effectively rendering the RBCs negative for such antigens. By this procedure we ensured that underlying alloantibodies such as anti-s, -s, -Fy\(^a\), -Fy\(^b\), -M, and -N remained behind after adsorption. We acknowledge that many workers have found that the s antigen is not readily destroyed.\(^7\) Although not every batch of reagent RBCs is specifically tested for antigen destruction, the process was thoroughly validated when introduced by the UK NBS reagents unit. Although no patients in this study were found to have underlying anti-s at the time of collating data, the patient referred to in Table 2 with anti-C, -Fy\(^a\), and -Jk\(^b\) did subsequently produce an underlying anti-s. The enzyme treatment usually increases the uptake and removal of autoantibody, although there were some exceptions to this in our study (four patients) when autoantibody removal was less effective.

Our standard protocol does not include the use of e– adsorption RBCs. In our experience the benefits of including RBCs of the R\(_2\)R\(_2\) phenotype are outweighed by the practical difficulties of obtaining suitable RBCs

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**Table 1.** Underlying alloantibody specificities

<table>
<thead>
<tr>
<th>Antibody specificity</th>
<th>Number identified</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-E</td>
<td>19</td>
</tr>
<tr>
<td>Anti-D</td>
<td>9</td>
</tr>
<tr>
<td>Anti-C</td>
<td>7</td>
</tr>
<tr>
<td>Anti-c</td>
<td>6</td>
</tr>
<tr>
<td>Anti-S</td>
<td>5</td>
</tr>
<tr>
<td>Anti-Fy(^a)</td>
<td>3</td>
</tr>
<tr>
<td>Anti-Jk(^a)</td>
<td>2</td>
</tr>
<tr>
<td>Anti-Jk(^b)</td>
<td>2</td>
</tr>
<tr>
<td>Anti-K</td>
<td>2</td>
</tr>
<tr>
<td>Anti-Kp(^a)</td>
<td>2</td>
</tr>
<tr>
<td>Anti-Fy(^b)</td>
<td>1</td>
</tr>
<tr>
<td>Anti-C(^w)</td>
<td>1</td>
</tr>
<tr>
<td>Anti-N</td>
<td>1</td>
</tr>
<tr>
<td>Anti-f (ce)</td>
<td>1</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>61</strong></td>
</tr>
</tbody>
</table>

**Table 2.** Multiple alloantibody specificities

<table>
<thead>
<tr>
<th>Antibody specificity</th>
<th>Number of samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-D,-C</td>
<td>3</td>
</tr>
<tr>
<td>Anti-c,-E</td>
<td>3</td>
</tr>
<tr>
<td>Anti-E,-Kp(^a)</td>
<td>1</td>
</tr>
<tr>
<td>Anti-E,-Fy(^a),-Jk(^a)</td>
<td>1</td>
</tr>
<tr>
<td>Anti-S,-N</td>
<td>1</td>
</tr>
<tr>
<td>Anti-Fy(^b),-f(ce)</td>
<td>1</td>
</tr>
<tr>
<td>Anti-c,-K,-Fy(^a)</td>
<td>1</td>
</tr>
<tr>
<td>Anti-C,-Fy(^a),-Jk(^b)</td>
<td>1</td>
</tr>
<tr>
<td>Anti-D,-C,-E,-S</td>
<td>1</td>
</tr>
<tr>
<td>Anti-E,-K,-Jk(^a)</td>
<td>1</td>
</tr>
<tr>
<td>Anti-C,-Jk(^b)</td>
<td>1</td>
</tr>
</tbody>
</table>
and the increased volume of plasma required. However, RBCs of the R2R2 phenotype are used when the initial investigation suggests they may be useful. The policy of matching the Rh phenotype of the donor units to patients with autoantibodies decreases the risk of an underlying alloanti-e causing a transfusion reaction.

Recently transfused patients may give misleading or inconclusive Rh phenotyping results. In such cases it is important to establish the transfusion history and make an individual assessment of the most appropriate Rh phenotype to select. Where possible it is good practice to prospectively establish the extended phenotype of patients likely to be multiply transfused.

Alloadsorption procedures carry the risk that an alloantibody to a high-prevalence blood group antigen can be adsorbed by the procedure and not detected when screening or matching blood. We believe our findings show that it is better to carry out the adsorption procedures, being aware of this risk, than not to adsorb at all. We have experience of one case (not included in this study) where an underlying anti-Vel was not detected following alloadsorption (data not published).

2. Autoadsorption

Autoadsorption uses the patient’s own RBCs to remove autoantibody so that underlying alloantibodies can be tested for. The advantages of autoadsorption are that it does not remove any alloantibodies and it requires adsorption of only a single aliquot of patient’s plasma, considerably reducing the volume required. The number of tests performed is also reduced since the tests need not be carried out in duplicate, or triplicate as in the case of alloadsorptions.

Autoadsorption is not without its problems, however. The patient’s RBCs often are heavily coated with antibody, with reduced capacity for further antibody uptake. Patients may also be anemic, sometimes very anemic, with few RBCs available for autoadsorption. In our experience, anything less than two equal-volume (RBCs:plasma) adsorptions does not remove sufficient autoantibody to be of investigative value. The relatively large amount of plasma available from anemic patients lends itself more to alloadsorption.

Autoadsorption is inappropriate for the recently transfused patient. If the procedure is inadvertently applied to samples from such patients there is a risk that alloantibodies may be adsorbed in vitro onto transfused RBCs. Our protocol for patients transfused within the last 3 months is to perform alloadsorption and to prepare an eluate to detect alloantibodies bound in vivo.

Transfusion practices

Transfusion in the presence of panreactive warm autoantibodies can be a complicated and dangerous proposition. In our study, adsorption techniques excluded the presence of alloantibodies in 69 percent of our patients. In the patients found to have alloantibodies, adsorption studies allowed the selection of appropriate RBCs for transfusion. In this group, Rh and Kell phenotype matching alone would have exposed 14 patients (11%) to the risk of a significant transfusion reaction.

We believe this study reinforces the value of adsorption studies when panreactive warm autoantibodies are present and concurs with the findings of similar studies. Our findings also show that it is not appropriate in these cases simply to issue RBCs that are “least incompatible” or Rh phenotype- and K antigen-matched.

References

Alloantibodies underlying autoantibodies


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Persistent anti-Dr\textsuperscript{a} in two pregnancies


The Drori (Dr\textsuperscript{a}) antigen is one of the ten high-prevalence antigens of the Cromer blood system, which are carried on decay-accelerating factor (DAF; CD55). The Dr(a–) phenotype was first described in a 48-year-old Jewish woman from Bukhara. Her serum contained an antibody to a high-prevalence antigen named anti-Dr\textsuperscript{a}. Most known individuals with the Dr(a–) phenotype are Jews from the geographic area of Bukhara, but individuals from Japan have also been described. Antibodies in the Cromer blood group system, including anti-Dr\textsuperscript{a}, have never been reported to cause HDN. In most of the cases with anti-Dr\textsuperscript{a} examined in Israel, the antibodies have been subtyped as IgG2 and IgG4. This report is of a woman with Dr(a–) phenotype and an anti-Dr\textsuperscript{a} titer of 256 to 512 in her serum, observed during two successive pregnancies. At birth, the RBCs of the first- and second-born child were negative and positive in the DAT, respectively, and neither manifested clinical signs of HDN. The disappearance of Cromer system antibodies, including anti-Dr\textsuperscript{a} in midpregnancy, has been described in a previous study. In that study, it was theorized that the antibodies in the serum of the women were adsorbed onto placental DAF. The finding of a high anti-Dr\textsuperscript{a} titer in two successive pregnancies in this patient, with a positive DAT for the RBCs of one of the two babies at term, differs from published reports, suggesting that a different mechanism might be involved. *Immunohematology* 2005;21:126–128.

**Key Words:** Anti-Dr\textsuperscript{a}, titers in pregnancy

The Drori (Dr\textsuperscript{a}) antigen is part of the Cromer blood group system, which includes ten high- and three low-prevalence antigens.\textsuperscript{1} The Cromer antigens are carried on the complement regulatory glycoprotein decay-accelerating factor (DAF; CD55). The Dr(a–) phenotype was first described in a 48-year-old Jewish woman of Bukharian origin by Levene et al.\textsuperscript{2} More cases have been published; most of them were of Jewish women born in Bukhara, a few were of individuals from Japan.\textsuperscript{3,4} Antibodies in the Cromer system have not been reported to cause clinical HDN.\textsuperscript{1} There are reports in the literature of women with Cromer antibodies in which the titer fell during pregnancy and the baby carrying a Cromer antigen was born with a negative DAT.\textsuperscript{5,6} DAF is expressed strongly on the apical surface of trophoblasts, more so in the second and third trimesters.\textsuperscript{7} The placental trophoblasts possess the DAF polymorphism of the fetus, which is inherited from both parents and is most likely positive for the high-prevalence Cromer antigens, including Dr\textsuperscript{a}. Reid et al.\textsuperscript{8} suggested that antigen-positive trophoblasts may absorb maternal antibodies with DAF specificity such as anti-Dr\textsuperscript{a}, causing their disappearance from maternal blood and explaining the lack of HDN secondary to this antibody. They reported two cases with Cromer antibodies, one of them anti-Dr\textsuperscript{a}, which disappeared during pregnancy, supporting their hypothesis.

In this report two successive pregnancies in a Dr(a–) woman with anti-Dr\textsuperscript{a} in her serum are described. The anti-Dr\textsuperscript{a} titers did not change significantly throughout either pregnancy. At birth, a DAT performed on the RBCs of the first baby was negative and that performed on those of the second baby was positive. Neither baby had clinical evidence of HDN.

**Case Report**

A 28-year-old Jewish woman of Bukharian origin in her second pregnancy was admitted to the Assaf Harofeh Medical Center delivery room in labor in March 1998. A RBC sample was sent to the blood bank for blood type and antibody screen. An antibody to a high-prevalence antigen was found in her serum and investigated. Her RBCs were typed as Group A\textsuperscript{1}, D\textsuperscript{1}, C\textsuperscript{1}, E\textsuperscript{1}, c\textsuperscript{1}, e\textsuperscript{1}, C\textsuperscript{w}–; M\textsuperscript{1}, N\textsuperscript{1}, S\textsuperscript{1}, s\textsuperscript{1}; P\textsubscript{1}–; Lu(a–b+); K\textsuperscript{1}, k+, Kp(a–b+); Le(a–b+); Jk(a+b); Yt(a+), and Dr(a–) and the DAT was negative. During testing for the Cromer antigens, weaker reactions were observed with anti-IFC and -Cr\textsuperscript{a} when compared with the positive controls. A sample from her husband was tested and his RBCs were determined to be Group A\textsuperscript{1}, D\textsuperscript{1}, C\textsuperscript{1}, E\textsuperscript{1}, c\textsuperscript{1}, e\textsuperscript{1}, C\textsuperscript{w}–; M\textsuperscript{1}, N\textsuperscript{1}, S\textsuperscript{1}, s\textsuperscript{1}; P\textsubscript{1}+; Lu(a–b+); K\textsuperscript{1}, k+, Kp(a–b+); Fy(a+b+); Jk(a+b–); Yt(a+b–); and Dr(a+). His antibody screen and DAT were negative.

The patient was found to have anti-D (low titer from passive immunization in pregnancy) and anti-Le\textsuperscript{H}. An antibody to a high-prevalence antigen was found in her serum, which was characterized as anti-Dr\textsuperscript{a}, and her RBCs typed as Dr(a–). The titer of the antibody by

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*IMMUNOHEMATOLOGY, VOLUME 21, NUMBER 3, 2005*
saline indirect antiglobulin test was 512 (Table 1). The patient’s serum was examined with two known Dr(a−) RBCs and the presence of anti-K, anti-M, anti-S, and anti-P1 were excluded.

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Materials and Methods

Standard tube hemagglutination tests were used for antigen typing, antibody screening, and panels. Antibodies and rare RBCs used in the testing were from the Israeli National Blood Group Reference Laboratory and SCARF. Column agglutination tests (DiaMed AG, Switzerland) were also used for antibody screening and panels. The RBCs were examined with anti-DAF (CD55) and anti-MIRL (CD59) in column agglutination tests (DiaMed AG). The elution was performed using the rapid acid method (Elukit II, Gamma Biologicals, Inc., Houston, TX).

Results

The RBCs of the newborn were negative in the DAT and there was no clinical evidence of HDN. In May 2000, during her next pregnancy, anti-Drα was identified with a titer of 256 (Table 1). In June 2000, the anti-Drα titer was 512 and in July 2000 the titer was 256. The pregnancy was uncomplicated and RhIG was again administered antenatally. The patient gave birth in October 2000 to a full-term healthy baby, the titer remaining stable throughout pregnancy. The infant’s RBCs were typed as Group A, D+; the DAT was positive with anti-IgG resulting in 2+ to 3+ reactivity in tubes and 4+ by the column agglutination test (DiaMed AG). The positive DAT precluded Drα typing. An elution was performed on the baby’s RBCs and anti-Drα was identified. The eluate did not react with RBCs from the mother, Dr(a−) RBCs, or RBCs treated with alpha-chymotrypsin. There was no clinical evidence of HDN. The titer of the anti-Drα in the mother’s serum was 256.

Testing of maternal RBCs by the column agglutination test (DiaMed) was negative with anti-DAF (CD55) and positive with anti-MIRL (CD59).

Discussion

DAF is a protein connected to the membrane via a glycosylphosphatidylinositol anchor. It protects cells against destruction by autologous complement by inhibiting formation and acceleration of the decay of C3 and C5 convertases, thus preventing the complement cascade from causing hemolysis. DAF is a member of the regulators of complement activation gene family encoded by a gene on the long arm of chromosome 1. It is widely distributed throughout the body and expressed on epithelial cells, endothelial cells, blood vessels, and the apical surface of trophoblasts. The Cromer antigens are carried on DAF and the different phenotypes provide a basis for biochemical and functional investigation of alternate forms of DAF. Dr(a−) is a rare Cromer phenotype, lacking expression of the Drα antigen, with reduced levels of DAF and weak expression of other Cromer antigens, including Crα, Tcα, Tcβ, Tcδ, Esα, IFC, Wesβ, and UMC. No hematological abnormalities have been described in Dr(a−) individuals. The molecular change associated with Dr(a−) has been characterized. Four pregnancies in a woman who had Cr(a−) RBCs and anti-Crα, in which the titer of the anti-Crα declined during pregnancy and remained low until term, were described by Sacks and Garratty. The decline in the titer was thought to result from absorption of the antibody by white cells or platelets or be due to neutralization by plasma of the fetus. Another case of anti-Crα in a pregnant woman in which the titer of the anti-Crα decreased was described by Dickson et al. Reid et al. described two patients with repeat pregnancies who had Cromer blood group system antibodies (anti-Crα and anti-Drα) with titers of 128 or greater at the beginning of pregnancy. These antibodies became undetectable in the serum from the second trimester onward. RBCs from the babies tested positive for the relevant Cromer antigens but were negative by the DAT. The antibodies would reappear in the serum of the mother after delivery or at the beginning of a subsequent pregnancy.

Holmes et al. showed the preferential expression of DAF at the feto-maternal interface of the placenta, increasing quantitatively during placentation development. DAF at this site plays a protective role against maternal complement-mediated attack. DAF can absorb the Cromer antibodies produced by the mother, more so in the second and third trimesters when the expression of DAF increases. This hypothesis is supported by reappearance of these antibodies after
pregnancy when the placenta is no longer present. Most of the patients with anti-Dr\(a\) found in Israel have been multiparous women. Subclasses of IgG examined in these cases during the years 1989–1991 were IgG2 and IgG4. The anti-IgG subtype antibodies used were from the Netherlands Red Cross and tests were performed in tube or capillary tests. None of the offspring had HDN (C. Levene, unpublished data, 1989–1991). The patient reported here was Dr(a–). She developed anti-Dr\(a\) and was monitored during two subsequent pregnancies. The anti-Dr\(a\) titer remained consistently at 256 or above during both pregnancies. RBCs from the first baby typed as Dr(a+) and the DAT was negative. RBCs from the second baby were positive in the DAT; they could not be typed for the Dr\(a\) antigen, but anti-Dr\(a\) was identified in the eluate, with no clinical signs of HDN. Our findings differ from previous\(^{5,6,8,11}\) reports, where anti-Cromer antibody titer declined during pregnancy. Reid et al.\(^8\) suggested that the titer decreased due to Cromer blood group system antibodies binding to the placental DAF. In the case described here, presumably the anti-Dr\(a\) was not adsorbed by the placental DAF, which might happen if the placental DAF was Dr(a–), if Dr\(a\) was poorly expressed, or due to another unexplained mechanism.

**Acknowledgment**

The authors wish to thank Leah Mendelson and Lydia Yosephi for technical assistance.

**References**

3. Levene C, Harel N, Kende G, et al. A second Dr(a–) proposita with anti-Dr\(a\) and a family with the Dr(a–) phenotype in two generations. Transfusion 1987;27:64-5.

_Naomi Rahimi-Levene MD, Director of the Blood Bank; Abram Kornberg MD, Chief of the Hematology Institute; Gabriela Siegel, High Risk Pregnancy Unit; and Valery Morozov, MD, Blood Bank, Assaf-Harofeh Medical Center, Zerifin, 70300, Israel; Eilat Shinar MD, Director; Orna Asher Phd, Director Immunohematology Laboratory; Cyril Levene MD, Consultant National Blood Group Reference Laboratory (NBGRL); and Vered Yahalom MD, Deputy Director & Medical Director NBGRL Magen David Adom (MDA)-National Blood Services, Ramat Gan, Israel._
COMMUNICATIONS

Letter to the Editors:

Inaugural meeting of the Consortium for Blood Group Genes (CBGG): a summary report

Over half a century ago the serologic analysis of blood group antigens established its roots in the clinical transfusion laboratory. The evaluation and laboratory investigation of alloimmunization, which occurred as a result of pregnancy or after RBC and platelet transfusion, was quickly developing into the field of immunohematology. Moreover, blood group phenotyping became a powerful tool to provide antigen-matched RBCs, to evaluate the differences in blood group antigen expression between individuals, and to infer the inheritance of the genes that express these antigens. Of course, a natural progression of the latter included the analysis of the genes and alleles that are responsible for the expression of the antigens. The ease with which molecular biological techniques became user friendly and adaptable to the clinical laboratories made this progression possible.

In 1993, Bennett and associates published a landmark paper on the clinical application of molecular blood group genotyping, although their initial application was not true “genotyping” as it merely confirmed the presence of the RHD gene. The unique application used DNA derived from cells in amniotic fluid to “infer” the blood group of fetuses at risk for HDN due to anti-D, thus alleviating the risk associated with cordocentesis to establish the D antigen of a fetus. Today, 50 percent of pregnancies at risk for the common antibodies implicated in HDN, when the father is heterozygous for the corresponding allele, are deemed to be antigen-negative and not at risk for the disease. Clinical management varies, but essentially the mother can return to her primary care physician and noninvasive procedures can be used to monitor for fetal anemia. A decade after the publication of Bennett et al., other molecular applications are being published with unprecedented speed. The DNA analysis of blood group genes has expanded to include the following uses:

1) Genotype multi-transfused recipients, patients with severe thrombocytopenia, and those whose RBCs or platelets are sensitized with antibodies
2) Resolve ABO and Rh D discrepancies
3) Identify some of the Rh D variants that are at risk for anti-D alloimmunization
4) Determine RHD zygosity
5) Confirm the true genotype when an antigen is weakly expressed, e.g., Del
6) Identify whether the Fy^b- transfusion recipient can safely receive Fy^b+ RBCs
7) Mass screen for antigen-negative and rare RBCs
8) Genotype donors for antibody identification panels

During the 1950s and 1960s people with a common interest in blood group serology formed focus groups to disseminate and exchange information, mentor young scientists, and advance our understanding of the field of immunohematology. So too has the study of the genes that express blood group antigens become an entity itself.

The inaugural meeting of the Consortium for Blood Group Genes (CBGG) was held during the AABB annual meeting on October 23, 2004, in Baltimore, Maryland. The meeting was attended by 24 delegates from North and South America, with contributions from other colleagues who could not attend. The group was formed out of these:

• A common interest in the genes that express RBC, platelet, and neutrophil antigens
• A desire to promote the use of molecular technology in the clinical lab
• The need for DNA reference samples
• The establishment of a proficiency program and standards of practice.

A summary of the meeting is provided in this report. All interested readers are encouraged to get involved and embrace the opportunity to discuss and promote scientific inquiry in blood group genes. It is anticipated that
clinical and scientific advancements in the study of the genes that express blood group antigens will continue as a result of global interaction. The next meeting will be held prior to the October 2005 AABB Annual Meeting in Seattle.

**Consortium for Blood Group Genes**

1) *Language*

Group meetings and interactions will be conducted in English.

2) *Membership*

Membership is open to those with an interest in single nucleotide polymorphisms and other genetic variations that are of interest to transfusion medicine, i.e., RBC, platelet, and neutrophil antigens. Interaction with other existing networking formats is encouraged, e.g., AABB Special Interest Groups.

3) *Name*

The name of the organization is the Consortium for Blood Group Genes (CBGG).

4) *Web Site*

A Web site will be established in the near future.

5) *Working Parties*

Working parties of volunteers were established to investigate, collate, and disseminate recommendations to the entire group on the areas listed below.

* a. *Proficiency Program*

One role of the CBGG is to contribute to and participate in proficiency testing much like immunohematology reference laboratories (IRLs), with two samples exchanged per year. One goal of proficiency testing is to have a forum for DNA testing verification and results analysis. The working party will recommend a practical, low-cost proficiency program.

* b. *Forms and Disclaimers*

Blood group genetic clinical reports were discussed and those members who provide clinical reports were asked to share copies of their reports to give a feel for the types of disclaimers they use. The group could help unify reports and set the standards for DNA blood group testing forms and reports and recommended disclaimers. The clinical forms and reports section will be addressed by a working party.

* c. *Reference DNA samples*

There is a need to establish a source of samples for control and for validation purposes. It is anticipated that standard DNA reference samples will be made available as well as a listing of important DNA single nucleotide polymorphisms, amplification primers, and standard protocols.

* d. *Standards*

The CBGG has the potential to develop standards for its members and act as a voice to regulatory bodies as the testing becomes more commonplace. An FDA representative has been contacted since the inaugural meeting. The group is encouraged to see an open partnership developing that will communicate needs and issues and ensure appropriate compliance.
e. Funding
Sources of funding were discussed. Possibilities included a nominal membership fee (e.g., $100), a fee for shipping DNA from the repository, and a fee for participating in the proficiency program. Other potential sources of funding were identified.

f. Structure and Bylaws
The structure, bylaws, and mechanism for nominating officers to represent the consortium are in the initial stages.

ERRATUM

Vol. 21, No.2, 2005; page 60

Review: acute Donath-Landsteiner hemolytic anemia

The author has informed the editors of Immunohematology that there is an error on page 60, Fig. 2. Set 3 in Fig. 2 should read “Normal serum.”

**Fig. 2.** Indirect Donath-Landsteiner test: sample conditions.
Monoclonal antibodies available at no cost. The Laboratory of Immunochemistry at the New York Blood Center has developed a wide range of monoclonal antibodies (both murine and humanized) that are useful for screening for antigen–negative donors and for typing patients' RBCs with a positive DAT. Monoclonal antibodies available include anti-M, -Fy\(^a\), -Fy\(^b\), -K, -k, -Kp\(^a\), -Js\(^b\), -Do\(^b\), -Wr\(^b\), and –Rh17. For a complete list of available monoclonal antibodies, please see our Web site at http://www.nybloodcenter.org/framesets/FS-4C7.htm. Most of those antibodies are murine IgG and, thus, require the use of anti-mouse IgG for detection, i.e, anti-K, -k, and -Kp\(^a\). Some are directly agglutinating (anti-M, -Wr\(^b\), and -Rh17), and a few have been humanized into the IgM isoform and are directly agglutinating (anti-Js\(^b\) and -Fy\(^a\)). The monoclonal antibodies are available at no charge to anyone who requests them. Contact: Marion Reid (mreid@nybloodcenter.org) or Gregory Halverson (ghalverson@nybloodcenter.org), New York Blood Center, 310 East 67th Street, New York, NY 10021.

**ANNOUNCEMENTS**

**Monoclonal antibodies available at no cost.** The Laboratory of Immunochemistry at the New York Blood Center has developed a wide range of monoclonal antibodies (both murine and humanized) that are useful for screening for antigen–negative donors and for typing patients’ RBCs with a positive DAT. Monoclonal antibodies available include anti-M, -Fy\(^a\), -Fy\(^b\), -K, -k, -Kp\(^a\), -Js\(^b\), -Do\(^b\), -Wr\(^b\), and –Rh17. For a complete list of available monoclonal antibodies, please see our Web site at http://www.nybloodcenter.org/framesets/FS-4C7.htm. Most of those antibodies are murine IgG and, thus, require the use of anti-mouse IgG for detection, i.e, anti-K, -k, and -Kp\(^a\). Some are directly agglutinating (anti-M, -Wr\(^b\), and -Rh17), and a few have been humanized into the IgM isoform and are directly agglutinating (anti-Js\(^b\) and -Fy\(^a\)). The monoclonal antibodies are available at no charge to anyone who requests them. Contact: Marion Reid (mreid@nybloodcenter.org) or Gregory Halverson (ghalverson@nybloodcenter.org), New York Blood Center, 310 East 67th Street, New York, NY 10021.

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3. Text
4. Acknowledgments
5. References
6. Author information
7. Tables—see 7 under Preparation
8. Figures—see 8 under Preparation

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   C. Running title of ≤ 40 characters, including spaces
   D. 3 to 10 key words

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   B. Purpose, methods, findings, and conclusions of study
   C. Key words—list under abstract

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   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:

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