Antibody Formation in Transfusion Therapy

Michael Passwater, MT(ASCP)SBB, DLM, CSSGB(ASQ)

ABSTRACT

The production of antibodies following blood transfusions is a complex process that involves many recipient and donor factors. Inflammation in the recipient is one important factor. As knowledge of the immune system, of oxygen, carbon dioxide, and nitric oxide pathways, and of hemostasis grows, more specific therapies will allow precise manipulation of the immune system and safer transfusions. Communication of patients' transfusion and immunotherapy histories with the laboratory, attention to detail in labeling pretransfusion specimens, checking patient and blood product identification before administration, and closely monitoring patients during transfusions remain critical to minimizing risks during transfusion therapy.

Key words: alloimmunization, antibody formation, blood administration, blood products, hemolysis, inflammation, patient safety, red blood cells, transfusion, transfusion reactions

The same immune system that protects people from invading microorganisms may also attempt to protect them from invading transfused or transplanted cells intended for therapy. While it is not known precisely who, how, and when antibodies will form in the transfusion setting, knowledge of the immune system is growing rapidly. As more details of the immune system's complexity, flexibility, and interactions within the body are discovered, new approaches can be implemented to enhance desired immune responses to destroy unwanted pathogens and diseased tissue, and to minimize unwanted immune responses, thereby preserving healthy tissues and transplants.

The body's largest defense mechanism is our skin and mucous membranes. In 1735, Benjamin Franklin wrote that an ounce of prevention is worth a pound of cure.[1] The importance of skin hygiene cannot be overstated. If the body's physical barriers are not able to keep an invader out, networks of complement and lectin proteins are the next line of defense within the body. Complement proteins may attach to cells and lyse them, mark cells to be phagocytized by immune cells, or activate immune cells to produce antibodies. Lectins are ubiquitous proteins in plants and animals. They may bind to carbohydrates and glycoproteins on bacterial cell membranes and influence cell recognition and adhesion. There is a growing appreciation for immune activation caused by pattern recognition sensors within tissues. These sensing mechanisms are termed *inflammasomes* and respond to both pathogen-associated molecular patterns and danger-associated molecular patterns. Both septic pathogen invasions and endogenous tissue inflammation, which may be termed *sterile sepsis* or *systemic inflammatory response syndrome*, may have similar impacts on host tissues including the immune system.[2,3] The *Danger Hypothesis* is a term used to convey the current understanding that the immune system responds to clues that an infection has taken place before responding strongly to antigens.[4] In other words, unless the immune system is first activated by inflammatory signals, foreign substances may be invisible to it. However, if the immune system is primed for action by inflammatory signals associated with intrinsic danger (damaged tissue) or extrinsic danger (pathogenic infections), then its cells will seek to engage and destroy cells displaying freign antigens. The human body contains roughly 60 000 miles of blood vessels. From major arteries to the smallest capillary beds, vascular endothelial cells line the interior of these vessels and actively maintain them. Recent evidence shows that damage to these endothelial cells may cause significant inflammation, activate and dysregulate the immune system, and contribute to the

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pathology of many diseases including sepsis, cardiovascular disease, diabetes, cancer, lupus, and especially sickle cell disease.[5] Additionally, endothelial inflammation plays a role in transfusion and transplant rejection by increasing the risk of alloantibody formation.[6]

ADAPTIVE IMMUNITY

Adaptive, or humoral, immunity is the most advanced immune response in mammals. It involves antibodies and the special cells and signaling proteins that influence them. Adaptive immune responses are complex, flexible, and interactive. The local environment and immune cells interact to recognize the situation, select a response to clear the problem, and rebuild healthy tissue.

The hallmark of adaptive immunity is the production of antibodies. There are 5 classes of antibodies: IgA, IgD, IgE, IgM, and IgG. IgA is typically found as a dimer in the mucous membranes of the gut, genitourinary tract, and bronchioles. IgD appears to mainly serve as a receptor on lymphocytes. These classes of antibodies are rarely involved in transfusion-related alloimmunization. IgE is involved in anaphylaxis, which is a significant and sometimes fatal risk of transfusion. Transfusion-related anaphylaxis is associated with proteins in donor blood that is infused into a recipient with a preexisting allergy, meaning the recipient lacks the protein and has been immunized from a prior exposure to the protein. An effective method to screen for anaphylaxis risk is not yet available.

Further discussion of IgA, IgD, and IgE is beyond the scope of this article. IgM and IgG are the classes of antibodies of most concern with transplants and transfusions. IgM antibodies are found within the intravascular space. They often hook together to form pentagons. The IgM pentagon is a larger complex that more effectively binds multiple antigens, attracts complement proteins, and may further influence immune cells. IgM is typically involved in the initial exposure to an antigen within the circulation. B cells with IgM antibodies that bind with the invading antigen will, with the proper environmental signals, rapidly divide and convert to production of IgG antibodies of the same specificity. Some of these B cells will become memory B cells, allowing a more rapid response to similar antigen exposures in the future.

IgG is evenly distributed throughout tissues and within the bloodstream. It is typically involved in secondary challenges (reexposure to an antigen). There are 4 subclasses of IgG antibodies. The subclass of IgG antibody and the amount and spatial proximity of antigen-antibody complexes that form influence whether or not complement proteins will attach to the IgG antibodies. In general, the more tightly packed the IgG antibodies are on a cell surface, the more likely complement proteins will attach to the antibody-antigen complexes. In addition to the density of the IgG antibody-antigen complexes present, the subclass also influences complement binding. IgG1 and IgG3 are much more efficient at binding complement than IgG2, and IgG4 rarely binds complement. The IgG4 subclass is often associated with immune tolerance after long-term exposures to an antigen. The IgG2 subclass is the least efficient at crossing the placenta, but the most efficient at responding to bacterial capsular polysaccharide antigens. IgG1 is the most abundant IgG subclass in most people, and it is the most proinflammatory of the 4 IgG subclasses.[7]

Each antibody has a Y-shaped appearance. The 2 *claws* on the Y contain variable and hypervariable regions. This end of the antibody molecule binds to the antigen. Antibody binding is a very 3-dimensional process, similar to fitting puzzle pieces together. The immune system attempts to select antibodies that are the best fit for the shape and electrostatic charge of the antigen(s) presented in proximity to tissue danger signals. The other end, or base of the Y-shaped antibody molecule, is the constant region. The constant region serves biologic functions such as complement attachment or activation of other immune cells (Figure 1).[8]

Lymphocytes are a class of white blood cells (WBCs) that have key roles in adaptive immune responses. B cells are a type of lymphocyte that mature into plasma cells, which then produce antibodies. Each B cell is programmed to produce antibodies with a single specificity. The antibody class produced may change (typically from IgM to IgG), but all antibodies produced by that cell and its offspring will have the same specificity. A healthy immune system has mechanisms to self-destruct any of its B cells producing antibodies that cross-react with healthy host cells.

T cells are another type of lymphocyte. T cells can be further subdivided into CD4 T helper cells and CD8 T suppressor cells. Cluster of differentiation (CD) receptor proteins are often used to identify and characterize immune cells. New subcategories of immune cells continue to be discovered. The influence of inherited differences in quantities and ratios of B and T cell subpopulations, as well as

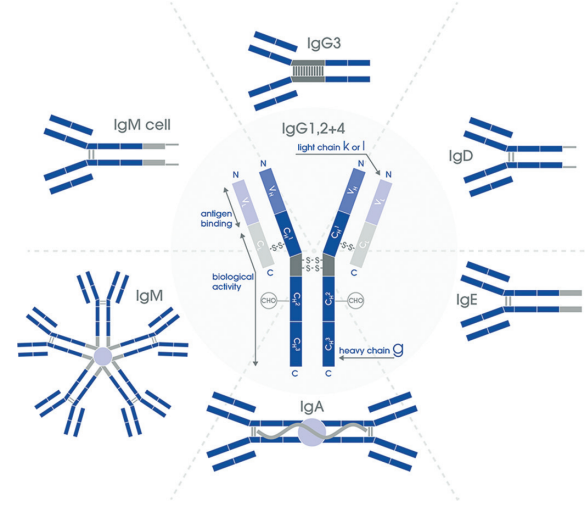

Figure 1 Antibody structures. Image provided courtesy of Abcam. Image copyright © 2018, Abcam. Used with permission.[8]

environmental factors impacting immune cell function, are active areas of research to better understand and control adaptive immune responses.

Antigen-presenting cells (APCs) are another category of cells in an adaptive immune response. The APC must display both a distress signal and a segment (antigen) of the invader to attract an activated T cell. The activated T cell then attracts a B cell with an antibody specificity that fits the invading antigen, and stimulates the B cell to divide and mass produce its antibody.[4] Figure 2[9,10] shows illustrations of APC, T cell, and B cell interactions necessary for antibody production. Dendritic cells have a major role in presenting antigens to T cells.[6] Monocytes and other T cells also serve as APCs. Even endothelial cells may serve as APCs in some environments with excessive inflammation.

In addition to these cells, adaptive immunity also relies on dozens of special signaling proteins. These cytokines, chemokines, neuroendocrine influences, and other cofactors present in the local environment direct cell receptor expressions, immune cell activations, movements, and responses.[11-13] The same immune cells may initiate, promote, prevent, suppress, or terminate an immune response depending on the signals they receive. Thus, the milieu of biochemicals surrounding the cells may be as important as the cells themselves to determine whether antibody production is triggered or silenced. The end result of a humoral (antibody) response is antibody coating of invading or inflamed cells, which may attract complement proteins to lyse the cells. Alternatively, the complement- or antibody-tagged cells may attract phagocytic WBCs within the circulation, or coated cells may be removed from the blood circulation in the spleen.

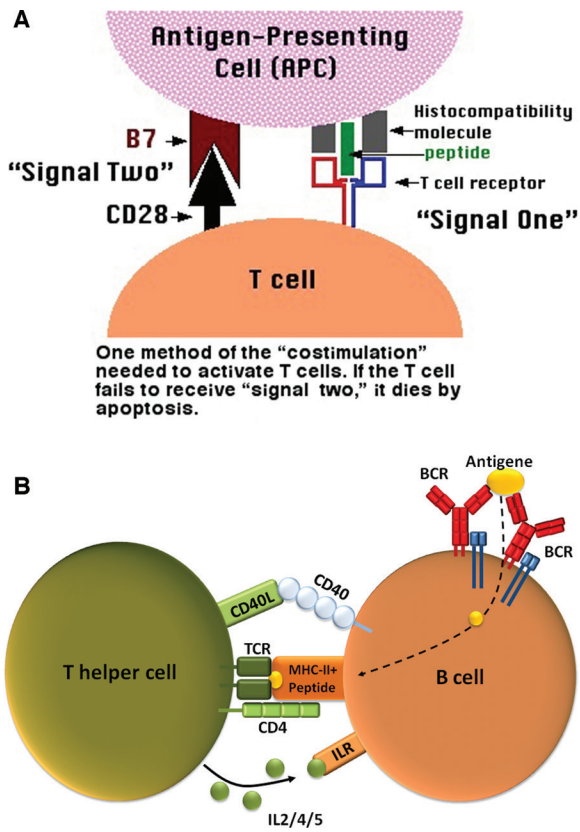

Figure 2 A) For antibody production to occur, a T cell must recognize both a foreign segment and a distress signal on the APC membrane,[9] and then B) find a B cell with an antibody to "fit" the foreign segment.[10]

The adaptive immune response is a powerful defense mechanism. However, the ability to mass produce antibodies to invaders that the body may never have seen before comes with risk. *Cross-reactivity* is a term used to describe antibodies selected for their specificity to 1 antigen (protein on a microbe or damaged cell) that also binds to similarly shaped antigens on unintended targets (healthy cells). If B cells that produce antibodies that cross-react with healthy tissue are not recognized and destroyed by regulator cells within the same immune system, damage to self-tissue (autoimmunity) may result. Interestingly, the brain, anterior chamber of the eye, and testes lack adaptive immunity. The risk of collateral damage from antibody production in these sensitive organs outweighs the benefits. As a result, tissue may be transplanted in these areas without the risk of humoral rejection.

ANTIGEN FACTORS INFLUENCING ANTIBODY PRODUCTION

In addition to activation of the immune system through danger signals, an antigen must also be present and perceived to be different from the host tissues to generate an adaptive immune response. It also must be large and spatially complex. Substances with molecular weights below 6000 daltons are not likely to stimulate an immune response unless they are attached to a much larger protein or cell. For size perspective: aspirin is 180 daltons, insulin is 6000 daltons, casein milk protein is 24 000 daltons, red blood cell (RBC) Kell antigen proteins are 93 000 to 115 000 daltons, and immunoglobulin G proteins are 150 000 daltons. Similarly, a simple straight-chain molecule such as a glucose polymer is unlikely to stimulate an immune response regardless of its size. The dose and route of entry also influence immune responses. Dose refers to both the time of exposure and the quantity of the foreign antigen present. The genetic background of the individual is also important. Genetics influences both the definition of self and the quantity and ratios of the numerous subcomponents of the immune system present.[14] Adding together these factors, it can be anticipated that a large substance different from the host, inside an inflamed area of the body for a significant amount of time, may draw the attention of an adaptive immune response (ie, generate antibody production).

RBC and platelet transfusions from community donors have much in common with the criteria necessary for antibody production. As for foreignness, humans have 8 major RBC types (O+, O−; A+, A−; B+, B−; AB+, AB−);

tens of thousands of different lymphocyte antigens (eg, A, B, C, DR, DQ, DP alleles of the human leukocyte antigen [HLA] system); hundreds of minor RBC antigens (eg, Rh, Kell, Kidd, Duffy, MNS, Lewis systems); and 2 dozen human platelet antigens (HPA system). As for size and complexity, RBCs, WBCs, and platelets are as large or larger than most invading microbes. As for dose, a typical healthy adult has a blood volume of 5 liters, or 10 pints of whole blood. This includes 20 to 30 trillion RBCs. Each pint, or unit, of donated blood is expected to contain 2 trillion RBCs and 1 billion WBCs. After leukoreduction, the unit may still have a million WBCs. A single-donor apheresis unit of platelets has 300 billion platelets per bag. Each RBC from a group A donor expresses about 1 million A antigens. With 2 trillion RBCs per bag, that means each bag has 2 trillion million, or a quintillion (10^{18}), A antigens. Similarly, the main Rh system antigen (D) is expressed approximately 20 000 times on each Rh+ RBC, and the main Kell system antigen (K or K1) is expressed approximately 5000 times per RBC.[15,16]

In addition to volume, time is also an important component of an antigen's dose. RBCs typically circulate for approximately 120 days in the human body. Therefore, RBCs may circulate for weeks to months after transfusion in a nonbleeding recipient. However, in a rapidly bleeding surgical patient, the transfused cells may flow through the patient in minutes to hours, not allowing time for a primary adaptive immune response. As for route of exposure, venous infusions bypass most innate immune mechanisms. With all those cells and antigens going directly into a person's veins, it is critically important to make sure patient and donor identification is complete and accurate for all blood bank testing and blood administrations. It is also understandable why there is growing popularity for the concept of viewing blood—or more comprehensively, the vasculature—as an organ, and replacing the term *blood transfusion* with *blood transplant*. RBCs, WBCs, platelets, plasma, and vascular endothelial cells comprise the vasculature and are critical to hemostasis and the regulation of the 3 gases of life: oxygen (O_2), carbon dioxide, and nitric oxide (NO).[17] Arguably, these blood vessels and the fluid they contain are as important as any solid organ.

Clearly, donor RBC and platelet units meet the criteria for becoming a target of an adaptive immune response. As discussed previously, inflammation appears to also be a necessary ingredient for alloimmunization. There is great overlap between patients with inflammation and patients receiving transfusions. There are indications that endothelial cell inflammation is especially hazardous to overall well-being and is a potent activator of the adaptive immune response. Free hemoglobin is especially toxic to vascular endothelial cells. Once outside the protective RBC cell membrane, hemoglobin rapidly oxidizes. The proteins haptoglobin and hemopexin circulate in plasma to scavenge free hemoglobin before it damages endothelial cells. However, it only takes a few milliliters of hemolyzed RBCs to overwhelm available supplies of haptoglobin and hemoplexin.[18,19] Conditions such as sickle cell anemia, autoimmune hemolytic anemia, systemic lupus, and chronic dialysis are associated with hemolysis, which leads to chronic endothelial inflammation, which then increases the risk of alloimmunization when these patients receive RBC or platelet transfusions. However, even in sickle cell disease, fewer than half (18%-47%) of transfused patients appear to form RBC alloantibodies.[20,21] There is still much to learn about antibody formation in transfusion therapy.

CURRENT ALLOIMMUNIZATION PREVENTION STRATEGIES

Avoiding transfusion is the one certain way to prevent transfusion-related alloimmunization. Conserving patients' blood; managing anticoagulation and antiplatelet medicines; and using hematinics such as intravenous (IV) iron, vitamin B_{12}, folic acid (vitamin B_9), and erythropoiesis-stimulating agents has been shown to reduce the need for blood transfusions in many settings.[22,23]

If RBC or platelet transfusion is necessary, using prestorage leukoreduced products reduces the risk of alloimmunization by both removing many WBCs (reducing the dose of WBC/HLA antigens) and reducing the quantity of inflammatory cytokines released from WBCs in the blood product bag during storage, thereby minimizing exposure to unwanted inflammatory proteins infused along with the desired blood product. When patients are likely to receive long-term RBC transfusion therapy, extended phenotype or genotype matching may be helpful. Matching additional minor antigens as well as the major ABO and Rh antigens minimizes the "foreignness" aspect of the infused donor cells.

Blood centers have also developed strategies to minimize harm from antibodies or other proteins present in the plasma of blood donors. The use of plasma products from male donors, or from female donors tested and found to not have HLA antibodies, has been associated with a reduction in transfusion-associated acute lung injury (TRALI) caused by passive immunity from donor HLA antibodies. Platelet additive solution products are also available. These products contain two-thirds less donor plasma than traditional platelet products suspended in 100% donor plasma and have been associated with fewer allergic reactions.[24]

LABORATORY TESTING

Complete and accurate patient identification on blood bank specimens and blood administration documents, careful testing within the laboratory, and a comprehensive history of treatments and previous blood bank workups are important to avoiding complications from RBC-related antibodies. Basic blood bank testing for transfusion recipients includes the blood type, antibody screen, and crossmatch

tests. Blood type testing determines whether the person's RBCs express the A, B, AB (both A and B), or O (neither A nor B) antigens, and whether or not the main Rh antigen D is expressed. If the D antigen is expressed, a person is considered Rh positive, and if it is not, she or he is considered Rh negative. The antibody screen test looks for antibodies in the person's plasma that react with RBC antigens other than the major A and B blood group antigens. If the patient's plasma contains antibodies that react with minor RBC antigens on the antibody screening cells, additional testing is required to determine the specificity of the antibodies, and donors lacking the antigens corresponding to the identified antibodies must be located to provide a safe RBC transfusion. Identifying the specificity of antibodies and finding compatible donors is typically the most time-consuming part of pretransfusion testing. Workups for patients with alloantibodies may take hours to days to complete. The crossmatch is a final check testing the patient's plasma against donor RBCs in the laboratory to verify that no lysis or agglutination occurs. Another test, the direct antiglobulin test (also referred to as a DAT or direct Coombs test), may be performed to see whether IgG or complement proteins are attached to RBCs in the patient's circulation. A positive DAT may indicate that alloantibodies, autoantibodies, or drug-induced antibodies are present. Figure 3 shows illustrations of blood bank testing for minor (non-ABO) RBC antibodies.

The goal of pretransfusion testing is to prevent transfusion reactions resulting from immune hemolysis. However, there are other adverse effects of transfusion that do not involve immune-mediated destruction of RBCs, and for which preventive testing does not exist. For instance, mild urticarial allergic reactions occur in 1% to 3% of transfusions. More severe anaphylactoid or anaphylactic reactions occur in 1 in 20 000 to 1 in 50 000 transfusions. Transfusion-associated circulatory overload (TACO) is estimated to occur in 1% to 8% of transfusions, TRALI may occur in 1 in 5000 transfusions, and septic reactions may occur in 1 in 75 000 platelet transfusions or 1 in 500 000 RBC transfusions.[25(p667-671),26] Tingling, numbness, and arrhythmias may occur as a result of electrolyte disturbances in the setting of large-volume transfusions due to citrate toxicity and/or potassium buildup in stored RBC units. As with all IV medication infusions, it is important to evaluate patients for fluid and electrolyte disturbances as well as other types of acute reactions.[27] With regard to immune-mediated hemolytic transfusion reactions, in spite of sensitive testing for RBC antibodies, acute hemolytic reactions are estimated to occur in 1 in 6000 to 1 in 40 000 RBC transfusions, and delayed hemolytic reactions may occur in 1 in 2500 to 1 in 11 000 RBC transfusions.[25(p667-671)]While there has been a downward trend in fatalities related to immune hemolysis reported to the US Food and Drug Administration since 2001, immune hemolysis continued to represent 21% of reported transfusion fatalities in the United States from 2010 to 2014. Of these immune-related fatality reports, the most frequently implicated antibodies were directed against antigens in the ABO system (34%), Kidd system (13%), and Kell system (10%).[29]

TRANSFUSION MONITORING, REACTION RECOGNITION, AND RESPONSE

Patient and blood product identification and patient monitoring are critical aspects of transfusion safety. Whenever possible, have a patient state his or her name and date of birth as part of the identification process. Be sure to check the patient and blood product identification at the bedside with another clinician, or with an electronic identification verification system approved for use with blood administration. It is also essential to take and document the patient's vital signs before, during, and after transfusion. Temperature, pulse, blood pressure, and respiration rate are the standard vital signs required to monitor with all transfusions; O_2 saturation is also monitored in some settings. There is much overlap in the signs and symptoms of different types of transfusion reactions. Therefore, further investigation of all possible transfusion reactions is required to evaluate causes. If new-onset adverse signs and symptoms such as fever (1°C increase and >38°C); rigors; respiratory distress; pain at the infusion site, flank, or elsewhere; gastrointestinal purging; or tingling and numbness occur during or shortly after (within 6 hours) transfusion, be sure to report the event to the patient's provider and the blood bank.

The initial steps for all suspected transfusion reactions are the same[30]:

- STOP the transfusion—many reactions are dose dependent.
- Maintain IV access—IV treatments may be necessary, so don't lose access.
- Recheck all patient and blood product identification—clerical errors remain the leading cause of ABO-incompatible transfusions.
- Notify the patient's provider and the blood bank.
- Support the patient's vital signs.
- Collect blood specimen(s) and observe or collect urine. No single test is diagnostic of TACO or TRALI. However, a chest x-ray as well as a B-type natriuretic peptide test to rule out cardiogenic pulmonary edema may be helpful in the evaluation of reactions involving respiratory distress.[25(p667-671)]
- Send blood product, IV administration set, and specimens to the laboratory.

Acute intravascular hemolytic reactions may occur minutes to hours after the transfusion begins and involve pain, fever, chills, hemoglobinuria (iced tea-, red wine-, or black-colored urine), and new or increased bleeding due to disseminated intravascular coagulation. Within the blood vessels, free hemoglobin binds NO irreversibly, which may

A

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al	RhHr	Donor	Rh Hr							Kell						Duf		Kid		Lew		P	MN				Lut		X		Ad A
			D	C	E	c	e	Cw	f	V	K	k	Kpa	Kpb	Jsa	Jsb	Fya	Fyb	Jka	Jkb	Lea	Leb	P1	M	N	S	s	Lua	Lub	Xga	Sex	
1	R1wR1	105125	+	+	0	0	+	+	0	0	0	+	0	+	0	+	+	0	+	0	0	+	+	+	+	+	0	0	+	+		4
2	R1R1	113990	+	+	0	0	+	0	0	0	+	+	0	+	0	+	+	+	+	+	0	+	+	0	+	0	+	0	+	0		4
3	R2R2	102333	+	0	+	+	0	0	0	0	0	+	0	+	0	+	0	+	+	+	+	0	+	+	+	0	+	0	+	+		4
4	Ror	116420	+	0	0	+	+	0	+	0	0	+	0	+	0	+	+	+	0	+	0	0	+	+	+	+	+	0	+	0		4
5	r'r	116457	0	+	0	+	+	0	+	0	0	+	0	+	0	+	+	+	+	0	0	+	+	+	+	0	+	0	+	+		4
6	r''r	117243	0	0	+	+	+	0	+	0	+	+	0	+	0	+	+	+	0	+	+	0	+	0	+	0	+	0	+	+		4
7	rr	114325	0	0	0	+	+	0	+	0	+	+	0	+	0	+	0	+	0	+	0	+	0	+	+	+	+	0	+	+		4
8	rr	106524	0	0	0	+	+	0	+	0	0	+	0	+	0	+	0	+	+	0	+	0	0	+	0	+	0	+	+	0		4
9	rr	116163	0	0	0	+	+	0	+	+	0	+	0	+	0	+	+	0	+	0	0	+	s	0	+	0	+	+	+	0		0
10	rr	112782	0	0	0	+	+	0	+	0	0	+	0	+	0	+	0	+	+	0	0	0	0	+	+	+	+	0	+	+		4
11	R1R1	117284	+	+	0	0	+	0	0	0	0	+	0	+	0	+	0	+	0	+	0	+	s	0	+	+	0	0	+	0		4

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ial	RhHr	Donor	Rh Hr								Kell						Duf		Kid		Lew		P	MN				Lut		X		Ad A
			D	C	E	c	e	Cw	f	V	K	k	Kpa	Kpb	Jsa	Jsb	Fya	Fyb	Jka	Jkb	Lea	Leb	P1	M	N	S	s	Lua	Lub	Xga	Sex	
1	RzR1	A2926	+	+	+	0	+	0	0	0	0	+	0	+	0	+	+	+	+	0	0	+	+	+	0	+	+	0	+	0		4
2	R1wR1	B2598	+	+	0	0	+	+	0	0	0	+	0	+	0	+	0	+	0	+	0	+	+	+	+	0	+	0	+	+		4
3	R2R2	C2158	+	0	+	+	0	0	0	0	0	+	0	+	0	+	+	0	+	+	0	+	+	+	0	0	+	0	+	+		4
4	Ror	D759	+	0	0	+	+	0	+	+	0	+	0	+	+	+	0	0	+	0	0	0	+	+	+	0	0	0	+	+		2
5	r'r	E550	0	+	0	+	+	0	+	0	0	+	0	+	0	+	+	+	+	0	+	0	+	+	+	+	+	0	+	0		4
6	r''r	F170	0	0	+	+	+	0	+	0	0	+	0	+	0	+	+	0	0	+	0	0	0	+	0	+	+	0	+	+		4
7	rr	G459	0	0	0	+	+	0	+	0	+	+	0	+	0	+	0	+	+	+	0	0	+	+	0	+	0	+	0	+	0		0
8	rr	H177	0	0	0	+	+	0	+	0	0	+	0	+	0	+	+	0	+	+	+	0	0	+	0	0	+	+	+	0		4
9	rr	N1524	0	0	0	+	+	0	+	0	0	+	0	+	0	+	0	+	+	+	0	+	w	+	0	+	0	0	+	+		4
10	R1R1	B3663	+	+	0	0	+	0	0	0	+	+	0	+	0	+	+	+	+	0	0	+	+	+	0	+	0	0	+	+		4
11	R2r	J1211	+	0	+	+	+	0	+	0	0	+	0	+	0	+	0	0	+	+	0	+	+	0	+	+	+	0	+	0		4

B

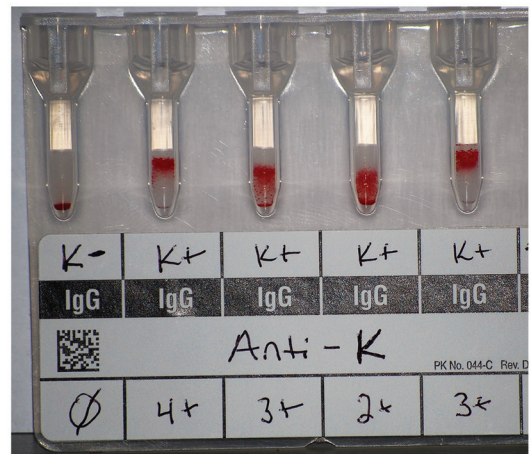

Figure 3 A) Illustration of blood bank testing to identify the specificity of "minor" (non-ABO) RBC antibodies. B) Illustration of blood bank test reactions using recipient plasma and donor or reagent red blood cells. 0 = Negative (no reaction), 4+ = strongly reactive (large agglutination/clumping). Image 3B provided courtesy of Ortho Clinical Diagnostics. Used with permission.

lead to vasoconstriction and shock. These reactions may be caused by IgM or high-titer IgG antibodies. Haptoglobin is typically undetectable. There is a risk of bystander hemolysis, also referred to as hyperhemolysis syndrome, meaning that the patient's own RBCs may be destroyed along with the incompatible donor RBCs, causing the posttransfusion hematocrit to be lower than the pretransfusion level. The combination of group A RBCs and group O plasma appears to be the most dangerous ABO incompatibility. This may be due to the large number of A antigens expressed on group A RBCs, and to the increased frequency of complement-binding anti-A,B antibodies found in group O donors (Figure 4).

Delayed extravascular hemolytic reactions typically occur 3 days to 3 weeks after transfusion. These reactions involve IgG antibodies and removal of incompatible antibody-coated donor RBCs by the spleen. The patient typically presents with jaundice from increased indirect bilirubin, and worsening anemia (decreased hemoglobin and hematocrit)—both a result of accelerated donor RBC removal and breakdown in the reticuloendothelial system (special cells in the spleen, liver, and lymph nodes that metabolize and recycle iron, hemoglobin, and other RBC components). The patient may have a positive DAT and/or a positive RBC antibody screen.

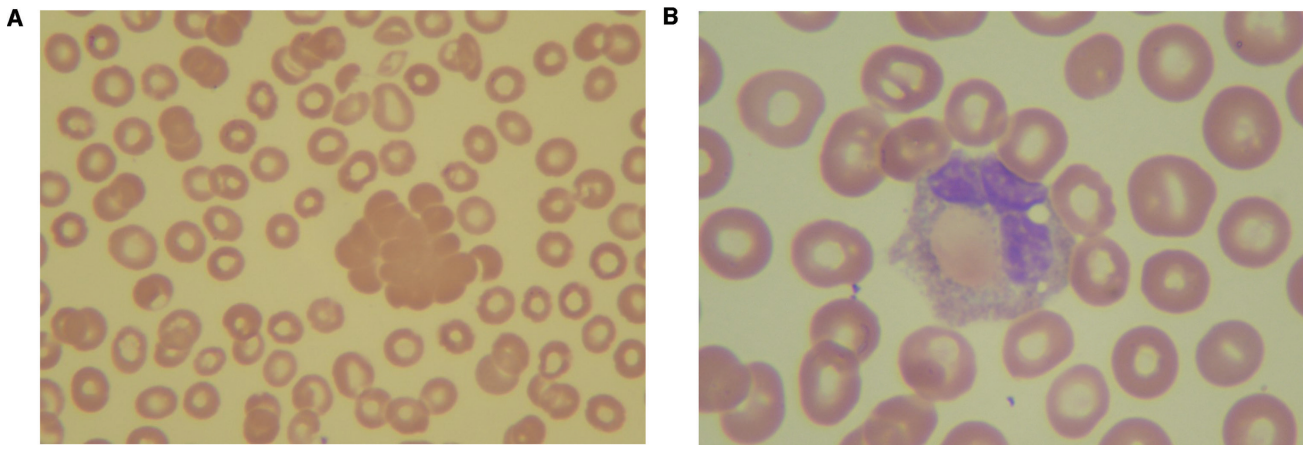

Figure 4 A) RBC agglutination and B) erythrophagocytosis. Simulated Group O recipient whole blood mixed with Group A RBC donor. Abbreviation: RBC, red blood cell.

ADDITIONAL ALLOIMMUNIZATION CHALLENGES

The main concern with alloimmunization in the setting of blood transfusions is harm to the transfusion recipient from acute or delayed immune hemolytic reactions. However, alloimmunization also causes testing delays and requires larger sample volumes for pretransfusion testing and more expensive workups. There is also an increased risk of future reactions due to unknown antibodies, or antibodies with decreased titers that are no longer detectable. A complete history of blood bank workups from a comprehensive medical history is important to ensure safe transfusions.

PASSIVE IMMUNITY

Rh immune globulin, intravenous immunoglobulin (IVIG), and some immunotherapies may contain antibodies that react with RBCs. It is helpful to notify the blood bank when patients are receiving these therapies to expedite workups and fully assess causes of hemolysis and anemia. Rh immune globulin is a concentrated preparation of anti-D and may also contain other Rh antibodies. IVIG contains anti-A and anti-B and may contain other RBC and HLA antibodies.[31] Daratumumab is a monoclonal anti-CD38 that may be given by IV infusion alone or with other medicines to treat multiple myeloma. The CD38 protein is heavily expressed on myeloma cells, but is also expressed to a lesser extent on many RBCs and other tissues.[32] Timely communication with the laboratory can minimize delays and confusion resulting from testing interference caused by these passive antibodies.

Rituximab is an anti-CD20 immunosuppressive therapy that binds with the CD20 protein on B cells and inhibits antibody production. It has been used successfully in autoimmune, transplant, and acute transfusion reaction settings.[33] Erlizumab is an anti-CD18 immunosuppressive therapy that targets lymphocytes and LFA-1 integrin to reduce inflammation. As more details of the immune system are discovered, more precise, selective therapies will be developed.

DRUG-INDUCED ANTIBODIES AND AUTOIMMUNITY

In addition to the formation of antibodies to transplanted RBCs, WBCs, and platelets, medications may also adhere to cells and generate adaptive immune responses as well.[34] The drug makes the cell appear foreign, and the cell makes the drug appear large to the immune system. If the immune system is in an activated state, antibody formation may result. Antibody formation may be directed strictly to the drug portion of the drug-cell complex, or it may be directed toward part of the cell membrane[25(p425-445),35] (Figure 5). Discontinuing the medication will eliminate the problem if the antibody is only directed against the drug portion of the drug-cell complex, but cell destruction may persist even if the drug is discontinued if antibodies to the cell membrane have formed. For example, heparin-induced

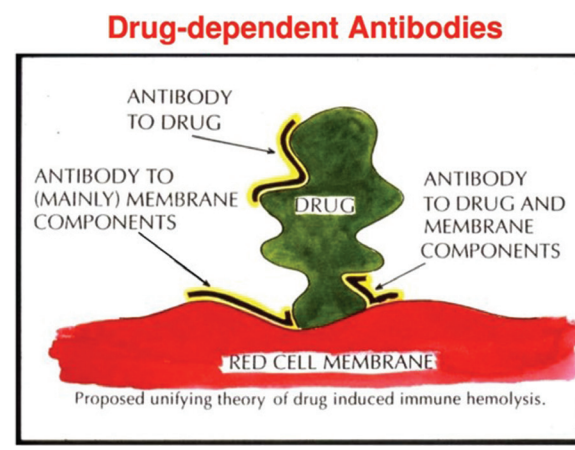

Figure 5 Proposed unifying theory of drug-induced antibody reactions based on a cartoon by Habibi as modified by Garratty.[35] Illustration used with permission from AABB.

thrombocytopenia (HIT) results from antibodies formed to heparin-platelet complexes. Low-molecular-weight heparin causes less HIT than the older, larger heparins. Antibiotics (especially cephalosporins and penicillins); platinum-containing chemotherapies; methyldopa; probenecid; and numerous other prescription, over-the-counter, and street drugs have been associated with antibodies that react with RBCs and platelets.

CONCLUSION

IV transfusion of foreign RBC, WBC, and platelet antigens into the presence of an activated immune response increases the likelihood of RBC, WBC, and platelet antibody formation. These alloantibodies may complicate current and future transfusions and may induce transient or chronic autoimmunity. Transfusion-associated immune destruction may manifest minutes to weeks after a transfusion begins. Attention to patient identification during pretransfusion specimen collection and blood administration, communicating and tracking patient transfusion histories and passive immunotherapies, and prompt recognition of reactions are critical to optimize transfusion safety. Future research will discover better ways to optimize wanted immune responses, minimize unwanted immune responses, and provide more specific therapies to minimize the need to transfuse foreign blood elements.

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