22 Transfusion Medicine and Immunohematology

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1. INTRODUCTION

Blood transfusion is essential and vital in the successful treatment of many malignant and nonmalignant hematological disorders. Children with thalassemia, adults with myelodysplastic syndromes, and patients with autoimmune hemolytic anemias, leukemias, or aplastic anemias become chronically dependent on blood transfusions. Modern treatment procedures such as high-dose chemotherapy and progenitor cell transplantation require intensive support with blood components and products. The serological basis of blood transfusion, the available blood

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components and products, and adverse effects of blood transfusion with special emphasis on infectious disease transmission are discussed in this chapter.

2. RED CELL SEROLOGY

2.1. The ABO, Hh, and Sese Systems

Genes for three different blood group systems (ABO, Hh, and Sese) indirectly control the expression of the A, B, and O antigens because antigenic activity is determined by sugars linked to either polypeptides (forming glycoproteins) or lipids (forming glycolipids). Each of the A, B, and H genes code for a specific enzyme (glycosyltransferase) that adds a different sugar on a polypeptide or lipid to form the ABH antigens.

The ABO system is the most important system in red cell serology and involves three allelic genes (A, B, and O) in chromosome a. The A and B genes encode glycosyltransferases (enzymes) that produce the A and B antigens, respectively. The O gene is considered to be nonfunctional because it determines no detectable blood group antigen. The expression of the O gene results in loss of production of a functional protein or enzyme; consequently, no product is formed. The red cells of a group O individual lack A and B antigens, but have an abundant amount of H antigen. The precursor H enzyme (fucosyltransferase) specifically adds fucose to a terminal galactose, thus giving H antigenic expression. If the glycosyltransferase adds *N*-acetyl-D-galactosamine to the terminal D-galactose of an H antigen, then the red cells will have the A antigen on their surface. Correspondingly, if the glycosyltransferase adds D-galactose, a B antigen is formed. ABO specificity is dependent on both ABO and Hh genes. Table 1 shows the incidence of the four main phenotypes of the ABO system.

The A blood group has two main subgroups: A_1 and A_2, which could be distinguished using the *Dolichos biflorus* lectin reagent. A_1 individuals have more A antigen sites than A_2 individuals. The A_2 gene differs from the A_1 gene by one base pair. Variability in genes causes variable reactivity as well add after "as well." Subgroups of B are very rare and less frequent than A subgroups.

In the ABO system, naturally occurring antibodies (isoagglutins) are present against A or B antigens. Individuals with the blood group A have isoagglutinins against B red cells and vice versa (*see* Table 1). These antibodies invariably are immunoglobulin (Ig)M that activate complement and cause immediate intravascular hemolysis resulting in severe acute hemolytic transfusion reactions (HTR). They are absent at birth and develop within 3–6 mo of age, when the immune system is exposed to ABH antigenic determinants present in our environment (i.e., bacteria, plants, dust, and food). Antibodies are naturally developed against the ABH antigens absent in the individual. Generally, the antibody titer increases until the age of 10 yr and progressively falls with increasing age in adults. In

Table 1
ABO Antigens and Antibodies (Isoagglutinins)

Phenotype	*Genotype*	*Antigens*	*Antibodies*	*Incidence (%)*[a] *Whites*	*Blacks*
O	OO	(H)	Anti-A Anti-B	45	49
A (A_1 or A_2)	AA or AO	A (A_1 or A_2) and (H)	Anti-B	40	27
B	BB or BO	B and (H)	Anti-A	11	20
AB (A_1B or A_2B)	AB	A, B, and (H)	None	4	4

[a]Incidence (%) in the United States among whites and blacks.

From Brecher ME, ed. *American Association of Blood Banks Technical Manual* (14th. ed.). Bethesda, MD: AABB Publications, 2002:p. 272.

acquired immunodeficiency states (e.g., leukemias and lymphomas) the levels may be significantly low.

The ABH antigens present in red cells have been demonstrated in most tissues of the body, including platelets and leukocytes. The ability to secrete soluble ABH antigens is controlled by the secretor (*Se*) gene that is separate from the ABH system. About 80% of the population have the dominant secretor (*Se*) gene that controls one's ability to secrete soluble ABH antigens. These individuals (secretors) distinctly have soluble ABH substances in their plasma and secretions (i.e., saliva, semen, and sweat).

2.2. The Rhesus System

The Rhesus (Rh) system is the second most clinically important and complex blood group system. It consists of some 50 different antigens, but only 5 antigens—D, C, c, E, and e—are inherited in various combinations and account for most of the Rh-related problems encountered in practice. The Rh antigen with the strongest antigenicity is the Rh (D) antigen. As a simple rule, it can be noted that persons whose red cells express the D antigen are Rh (D) positive and individuals whose red cells lack the D antigen are Rh (D) negative. The different genotypes, their Rh status, and the frequency of these genotypes in Caucasians are shown in Table 2. About 85% of North American Caucasians are Rh (D) positive.

After the discovery of the Rh system in 1940, various theories were postulated to explain the mode of inheritance and different nomenclatures were proposed. The Wiener system proposed that the gene product was a single entity with multiple serological specificities. The Fisher-Race system postulated three sets of closely linked genes and gene products (C and c, D and d, and E and e). Rosenfield proposed a third nomenclature system based on serological reactions, which assigns a number for each Rh antigen. The World Health Organization in

Table 2
Rhesus Genotype Frequencies and Their Rh Status

Genotype	*Rhesus status*	*Frequency*[a]
CDe/cde or R^1 r	Positive	32%
CDe/CDe or R^1R^1	Positive	16%
CDe/cDE or R^1R^2	Positive	11%
cDE/cde or R^2r	Positive	11%
Other genotypes with D	Positive	<13%
cde/ cde or rr	Negative	15%
Weaker variants of D or C	Positive for donors, negative for recipients	<1%
Rh-null	Null	Extremely rare

[a]In Caucasians.

1977 recommended the CDE nomenclature of Fisher-Race as it easily fits with the serological reactions. Cde or [R1] and cde or [r] are the most common haplotypes in Caucasians. cDe or [R^0] is most common in blacks.

Genomic studies have revealed the presence of two closely linked genes (*RHD* and *RHCE*) with considerable homology that refutes both Wiener and Fisher-Race postulates. The *RHD* gene controls the production of the D antigen and is absent in Rh (D)-negative individuals and explains the absence of the "d" antigen. The *RHCE* gene encodes for Cc and Ee antigens. The D and CE polypeptides differ in only 36 amino acids, the C and c polypeptides differ in four amino acids, and E and e differ in only one amino acid. The approximate molecular weight of a nonglycosylated Rh protein is 30 kDa. Recently, a D protein (a mixture of Rh [c] and [e]) has been isolated from Rh (D)-negative red cells that differ from the D protein of Rh (D)-positive cells. Genetic polymorphism may account for the difference.

Individuals whose red cells give weaker reactions with anti-D reagents are classified as *quantitative weak D* (red cells that require additional steps to demonstrate D were formerly classified as *D variant or D*^{u}). The D antigen has more than 37 epitopes, and if a significant number of epitopes are absent, then the individual is known to have *partial D* antigen (formerly classified *"D mosaic" or "D variant"*) and can produce an antibody to the portion of the D antigen they lack. *Partial D* phenotypes arise from nucleotide interchange between the *RHCE* and the *RHD* genes or from single mutations. Gene interaction also depends on the position of the genes that ultimately affect the expression of the D antigen. A *weak D* antigen can also result from the suppressive effect of ***C*** *in trans position to a* ***D*** *on the opposite chromosome* exemplified by *CDe/Cde.* A *weak D* individual because of gene interaction has the entire D antigen and can receive D-

positive blood. In contrast, *weak D* individuals who have partial absence of the D should only be transfused with D-negative blood because they can produce anti-D antibodies. The American Association of Blood Banks (AABB) requires that blood donors be screened for weak expression of the D antigen and to be labeled as Rh (D)-positive if the test is positive; however, recipients need not be tested for weak D.

On very rare occasions, red cells may lack the expected Rh antigens (e.g., –D–, –De, cD–). In Rh-null individuals, the Rh antigens are completely absent. This can arise from the absence of the gene that regulates Rh antigen expression or the presence of an amorphic gene at the Rh locus. Rh-null individuals have a compensated hemolytic anemia and abnormal red cell morphology (stomatocytosis). If transfused, they will produce antibodies against the different Rh antigens; therefore, Rh null individuals should be transfused only with Rh-null cells from the rare donor registry or with autologous red cells.

Rh antibodies can be acquired during pregnancy or a blood transfusion. The most common Rh antibody is anti-D. Rhesus immunization during pregnancy or delivery may occur when an Rh (D)-negative woman has an Rh (D)-positive child. This can be prevented by the prophylactic injection of anti-D Igs (RhIg). Rh antibodies are predominantly IgG and react at 37°C. They do not fix complement effectively, but can cause hemolytic disease of newborn (HDN; *see* Chapter 6) and hemolytic transfusion reaction (HTR). Extravascular hemolysis occurs through the mononuclear phagocyte system.

2.3. Other Red Cell Systems

Red cells bear antigens of many other blood group systems besides the ABO and Rh systems (Kell, Duffy, Lewis, I, P, MN, Lutheran, Kidd, and others). These red cell antigens are not routinely typed and generally are rare causes of HDN. The Kell and Duffy systems are briefly discussed. For more comprehensive information on the other red cell antigen systems, the reader is referred to reference texts. (*see* "suggested reading").

The Kell blood group system is clinically important, as the K antigen follows the D antigen in immunogenicity and its antibodies can cause HDN and HTR. Currently, this system includes 24 alloantigens, the most common being the K, k, Kp(a), Kp(b), Js(a), and Js(b) antigens. A defective and weak expression of Kell antigens (also lack Kx) is observed in individuals with the McLeod phenotype. These individuals have a chronic compensated hemolytic anemia and abnormal red cell morphology (acanthocytosis). Individuals with McLeod red cells also have neuromuscular and cardiovascular abnormalities (myopathy, areflexia, and cardiomyopathy). Rarely, the McLeod phenotype is associated with chronic granulomatous disease and arises from the deletion of the X chromosome that includes both XK and X-CGD loci.

The Duffy system is unusual in that the antigen frequency varies in different racial groups. The Duffy glycoprotein is the receptor for the malarial parasite and serves as an erythrocyte receptor for a number of cytokines, notably interleukin (IL)-8. Duffy glycoproteins also serve as a sponge for excess chemokines without any adverse effect on the red cell. This system has six antigens; two of these are important and deserve mention. Both Fy^a and Fy^b antigens have low incidence in Africans. In West Africa, most probably by natural selection, both antigens are absent in the majority of blacks [Fy (a–b–)]. Their red cells exhibit resistance to infection by *Plasmodium vivax* and *P. knowlesi*. Anti-Fy^a antibody may cause mild HDN and rare but severe HTR. Infrequently, Anti-Fy^b is associated with either HDN or HTR; other antibodies of this system have not been implicated at all.

2.4. Diagnostic Methods in Blood Group Serology

Prior to any blood transfusion, the red cell ABO and Rh(D) blood group (blood type) of the recipient is determined, and the serum is screened for any unexpected red cell antibodies (usually IgM and IgG antibodies). Thereafter, a cross-match is carried out between the donor's red cells and the recipient's serum. Blood group antigens or antibodies are determined with agglutination methods. The IgM antibodies (i.e., anti-A or anti-B) are usually detected by saline techniques, whereas enzyme, albumin, or antiglobulin methods are employed for the IgG antibody detection. Low ionic strength solution (LISS) is widely used in blood group serology as it shortens the incubation period and is helpful with emergency blood requests. The antiglobulin (Coombs') test detects antibodies coated on the red cells. The direct antiglobulin test (DAT) detects antibodies that are already bound to red cells in vivo, whereas the indirect antiglobulin test (IAT) detects antibodies present in the serum. The direct antiglobulin test may be positive in: (1) autoimmune hemolytic anemias (seen in lymphomas, system lupus erythematosus, cold agglutinin syndrome, and paroxysmal cold hemoglobinuria); (2) alloimmune hemolytic anemias (HDN and HTR); and (3) drug-induced hemolytic anemia. Figure 22.1 schematically outlines the determination of the ABO blood group with agglutination methods, whereas Fig. 22.2 shows the principles of both DAT and IAT.

3. LEUKOCYTE ANTIGENS

Human leukocytes bear two types of surface antigens: the human tissue or cell-specific antigens and individual type-specific antigens.

The first group is described in the cluster designation (CD) nomenclature. These antigens characterize the lineage, function, or activation state of the individual type of leukocyte (e.g., CD3 for mature T-cells). A list of the most current

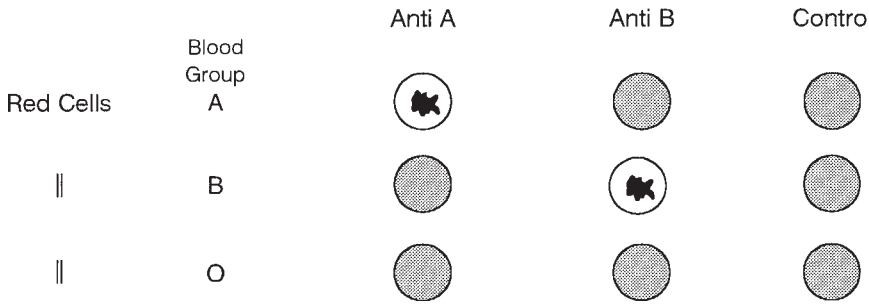

Fig. 22.1. Agglutination of red cells.

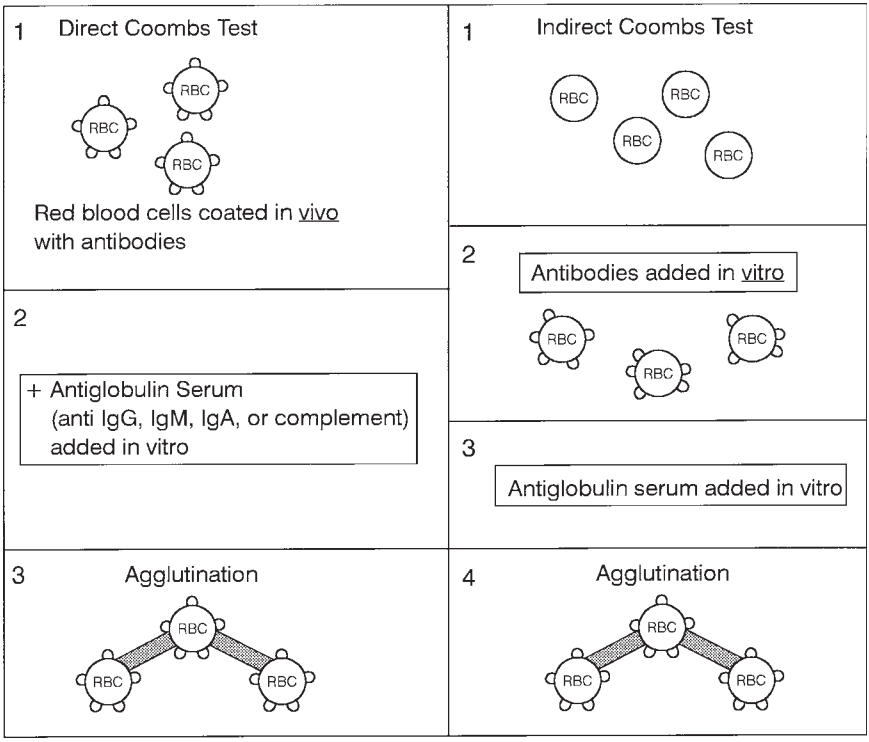

Fig. 22.2. Direct and indirect Coombs' test (antiglobulin test).

CD markers is given in Appendix 2. The second group, the family of human leukocyte antigens (HLAs) (Class I, II, and III antigens), is encoded by the major histocompatibility complex (MHC) genes on the short arm of chromosome 6. Class I and II antigens are the classic transplantation antigens that define tissue

tolerance or rejection and are important for organ transplantation, but their primary role is in immune response regulation. Class III antigens (i.e., complement C2 and C4 or tumor necrosis factor [TNF]-α) may be directly or indirectly involved with MHC function.

The HLA system is expressed on many tissues. The Class I antigens (HLA-A, -B, and -C molecules) are present on all nucleated cells and platelets. Class II antigens (HLA-D molecules) are expressed on B-lymphocytes, antigen-presenting cells (monocytes, macrophages, and dendritic and Langerhans cells), and activated T-lymphocytes. HLA Class I and Class II antigens differ in immunological function. Class I antigens interact with $CD8^+$ lymphocytes, which recognize endogenous antigens. Class II molecules on the surface of antigen-presenting cells bring exogenous antigens in contact with $CD4^+$ lymphocytes.

Class I molecules consist of two glycosylated heavy chains of 44–45 kDa and a noncovalently bound 12 kDa molecule (β_2-microglobulin). The class I heavy chain has three extracellular domains, a transmembrane region, and an intracytoplasmic domain. Each of the class II molecules (HLA-DR, DQ, and DP) consists of two transmembrane noncovalently associated glycosylated polypeptide chains. The α-chain has a molecular weight of about 30–34 kDa; the β-chain has a molecular weight of about 26–29 kDa.

The inheritance of the HLA genes is closely linked, and the entire MHC is inherited as an HLA haplotype (half of the genotype) in a Mendelian fashion from each parent. For example, a haplotype of *A3, B7, Cw7, and DR2* may come from the father and the other haplotype of *A9, B27, Cw1, and DR7* may come from the mother. Distances between loci may permit some chance of recombination within the HLA system, but this occurs only infrequently (<1%) and usually between DP and DQ loci. Statistically, siblings have a 25% chance of inheriting the same pairs of HLA molecules from the parents (i.e., being HLA identical).

The HLA system is the most polymorphic human antigen system, thereby showing an enormous number of different HLA haplotype combinations. The HLA antigen pattern varies among different ethnic groups. Because of linkage disequilibrium, some haplotypes occur more frequently in certain populations. For example, HLA-*A1, B8, DR3* is the most common HLA haplotype among Caucasians with a 5% frequency.

The HLA complex is divided into three regions indicating the locations of loci. Serological and DNA techniques differ in the number of alleles that they can identify for each locus. HLA-A, HLA-C, and HLA-B loci have 20, 8, and 30 serologically defined alleles but have 309, 167, and 563 alleles detected by DNA analysis. Among the Class II antigens, the HLA-DR, HLA-DP, and HLA-DQ molecules expressed on the cell membranes are most important with 442, 81, and

127 alleles defined by DNA techniques. The allelic variations of DQ-A, DP-A, and DP-B can only be defined by DNA methods.

Two HLA nomenclatures are currently in use. The older list of specificities is based on detection of epitopes by immunological techniques (serology or mixed leukocyte culture reactions) and the newer molecular nomenclature is based on specific nucleotide sequences of alleles using DNA-based methods, now a commonly used technique in HLA-typing laboratories. Peripheral blood lymphocytes express class I antigens and are used for the serological typing of HLA-A, HLA-B, and HLA-C. Class II antigens are typed using B-lymphocytes. Classic serology uses lymphocyte microcytotoxicity tests (by Terasaki) utilizing sera from multiparous women who have been immunized against certain HLA antigens. Clinical molecular techniques have revealed the complexities of both class I and II antigens. For example, the identities of class II antigens can be shown by the mixed lymphocyte reaction but HLA–D identical pairs remain nonreactive using this method. However, molecular typing is able to distinguish serologically indistinguishable but functionally discrete HLA alleles.

All current DNA-based HLA typing assays utilize PCR to amplify the genes of interest. There are three commonly used procedures: sequence–specific primers (PCR–SSP), sequence specific oligonucleotide probes (PCR–SSOP), and sequence-based typing (PCR–SBT). DNA typing is specific (no batch-to-batch variation in specificity) and flexible (new reagents can be designed as new alleles or new nucleotide sequences are identified). It is highly reproducible (with SSOP) and more robust than other techniques because it does not require viable lymphocytes nor is it influenced by the patient's health. DNA based methods have the added advantage of HLA-typing large numbers of volunteers for donor registries. Furthermore, it can detect the full range of HLA diversity. HLA alleles can specify the HLA proteins that are indistinguishable by serology. For example, *DRB1*0401 and DRB1*0412* are allele splits identified by DNA typing that belong to the broad specificity *DR4* serological type.

In addition to the major histocompatibility antigens just described, minor histocompatibility antigens (mHags) have been defined by both Class I and II MHC-restricted T-cells and may affect the outcome of progenitor stem cell and solid organ transplantation. The mHags are immunogenetic peptides bound to Class I molecules that stimulate T-cell responses. They are also inherited and the number of minor histocompatibility loci is probably high. To date, the range of polymorphism of these antigens is not well characterized. The disparity of mHags can be associated with graft-vs-host disease (GVHD) in HLA-identical transplants (i.e., H–Y antigen in a male recipient and a female donor immunized by pregnancy). The frequency of allelic forms, immunogenicity of peptides and tissue-specific expression of proteins will determine the role of mHag disparity in either GVHD or graft rejection.

3.1. Applications of HLA Testing

HLA testing is used in progenitor stem cell and organ transplantation, disease susceptibility studies, and parentage testing. HLA identity is the *sine qua non* of allogeneic bone marrow transplantation. Despite full HLA-identical progenitor cell grafts, a substantial number of patients develop graft-vs-host mHag reactivity (details about allogeneic progenitor stem cell and bone marrow transplantation are described in Chapter 4). The HLA antigens are also important for solid organ transplantation (i.e., kidney or liver). HLA-A, HLA-B, and HLA-DR are considered to be the major transplantation antigens, whereas HLA-C, HLA-DP, and HLA-DQ are generally of minor importance. In kidney transplantation, HLA matching between donor and recipient is done routinely. HLA-matched kidney grafts have better outcomes than unmatched grafts. In contrast to bone marrow transplantation, solid organ transplantation requires ABO-compatible grafts. For logistic reasons, other solid organs (heart, liver, lung, and pancreas) are not routinely matched for HLA-antigens. HLA antibodies play a major role in graft survival and chronic rejection. The presence of cytotoxic HLA antibodies in the serum of transplant recipients reactive against a panel of cells (expressed as panel reactive antibodies or [PRA]) lowers the graft survival rates and may be a contraindication for kidney transplantation. The PRA effect is greater among recipients of a second transplant.

Multiparous women and patients who receive multiple blood transfusions are frequently alloimmunized to HLA antigens. These HLA antibodies are broadly reactive. Post-transfusion HLA alloimmunization is variable and dependent on the patient's diagnosis and therapy. Patients with leukemia have lower detectable antibodies (25 to 30%) than patients with aplastic anemia (80%) because they are usually immunosuppressed from intensive chemotherapy when the transfusions are given. Leukocyte reduction of blood components to less than 5×10^6 has significantly reduced the development of primary HLA alloimmunization. This can be achieved by the use of third-generation leukocyte filters for red blood cells (RBCs) and platelets or by inline leukoreduction systems of blood cell separators used to collect pheresis blood components. As will be discussed later in the chapter, HLA antibodies have been implicated in febrile nonhemolytic transfusion reactions and transfusion-related acute lung injury.

Immunological refractoriness to platelet transfusions results from immune destruction of transfused platelets more often by HLA antibodies (Class I antigens are expressed on platelets) than by platelet-specific antibodies. Nonimmune causes of platelet refractoriness need to be ruled out such as splenomegaly, disseminated intravascular coagulation (DIC), bleeding, infection, marrow transplantation, and antibiotics (amphotericin B, vancomycin, ciprofloxacin). Detection of HLA- or platelet-specific antibodies is usually done by solid phase red cell adherence assay

(SPRCA), flow cytometry, ELISA, monoclonal antibody specific immobilization of platelet antigen assay, or mixed passive hemagglutination assay.

Once immune refractoriness is established, special platelet products are indicated. These patients can receive HLA-matched platelets, or platelet crossmatching can be done using SPRCA or flow cytometry. Both methods of crossmatching will detect platelet antibodies against Class I and platelet-specific antigens. The efficacy of crossmatched platelets may be as good as HLA-matched platelets. Platelet-crossmatched units have the additional advantage of being readily available for transfusion. Crossmatching is not always practical because alloimmunized patients may have HLA antibodies that react to more than 90% of the random population. These patients may also have few broadly reactive antibodies against public epitopes of Class I molecules, which will make it harder for a blood center to provide a product because the best HLA-matched platelet unit can still have some incompatibility.

Numerous diseases have a more or less strong association with certain HLA antigens. Well-known examples are ankylosing spondylitis associated with HLA-B27, narcolepsy associated with HLA-DR2, hemochromatosis associated with HLA-A3, celiac disease with HLA-*DQB1*02,* and type I diabetes mellitus with DR-3 and -4 heterozygotes. The *HLA-A1, B8, DR3* haplotype is frequently involved in autoimmune disorders. These disease associations indicate the central role of the major histocompatibility complex in determining the susceptibility to disease and immune responsiveness.

The HLA system is used in parentage testing because of its polymorphism with a low recombination rate and Mendelian inheritance. There is a decreasing use of HLA typing because it does not provide a high exclusion probability when a case involves a paternal haplotype common in one particular ethnic group. Thus, molecular techniques using non-HLA genetic systems are widely favored.

4. BLOOD PRODUCTS AND INDICATIONS FOR BLOOD TRANSFUSION

4.1. Whole Blood

The use of whole blood is limited and is indicated in massive blood loss to replace the loss of both RBC mass and blood volume. Many trauma centers have abandoned the transfusion of whole blood in favor of intravenous solutions in conjunction with RBCs and other blood components. Currently, whole blood units serve as source material for blood components and plasma products.

4.2. Red Blood Cells

RBCs are indicated for replacement of red cell mass in patients who require increased oxygen-carrying capacity to prevent tissue hypoxia. The hemoglobin

or hematocrit value, at which a transfusion is given, depends on the clinical circumstances. Current information supports a "restrictive strategy." Transfusions are indicated when hemoglobin concentration falls below 7 g/dL. The hemoglobin concentration should be maintained between 7 and 9 g/dL. **Note:** In younger patients, it may be necessary to transfuse if the hemoglobin concentration drops below 6 g/dL. In elderly anemic patients with cardiovascular disease (acute myocardial infarction and unstable ischemic syndromes), the threshold for transfusion may be 9 or 10 g/dL. To avoid volume overload, transfusions should be given slowly. In recent years, the recommended hemoglobin value for transfusion during surgery has been lowered from 10 to 7 g/dL. In a typical 70-kg (155-lb) patient, each unit of transfused RBCs is expected to raise the hemoglobin 1 to 1.5 g/dL and the hematocrit by 3 to 5%.

4.3. Platelets

Two types of platelet components are available for transfusion: "Pheresed Platelets," derived from single donors using an automated cell separator, and "Pooled Platelets," derived from whole blood donation and multiple donors. Automated cell separators effectively collect platelets (3 to 4×10^{11}/U) from donors.

The major goal of prescribing platelet transfusions is to effectively and safely prevent and/or treat bleeding in thrombocytopenic patients. Platelets may be given prophylactically in severely thrombocytopenic patients who have a hemorrhagic tendency or to patients on intensive myelosuppressive chemotherapy to keep the platelet count above 10×10^9/L. The success of modern chemotherapy in patients with hematological disorders (i.e., acute leukemias and myelodysplastic syndromes) and progenitor cell transplantation (bone marrow or stem cell transplantation) is largely dependent on effective platelet transfusions. Platelet counts for those at risk for spontaneous bleeding (patients with fever, infection, impaired platelet function from drugs, or hepatic or renal failure) should be kept above 15 to 20×10^9/L. Platelets are also indicated in other thrombocytopenic states: consumption coagulopathy, massive transfusion, GVHD, von Willebrand disease, and congenital and acquired platelet defects. Invasive procedures (i.e., lumbar puncture and liver biopsy) can be performed safely when the platelet count is at least 50×10^9/L. Counts of 100×10^9/L should be maintained if excessive bleeding cannot be tolerated (i.e., CNS or retinal procedures). In autoimmune thrombocytopenia, hypertransfusions of platelets are only indicated in cases of major hemorrhage. Platelets are not useful in most other instances, as the autoantibody shortens the survival of both transfused and patient's own platelets.

To investigate the mechanism of a poor response to a platelet transfusion, a platelet count is usually obtained within 1 h of the transfusion and the corrected

count increment (CCI) or percent recovery is calculated. Patients who are refractory to platelet transfusions (CCI < 7.5×10^9/L or percent recovery of <15 or 20%) may have a better response if transfused with a sufficient dose of apheresis platelets. For those who become immunologically refractory, HLA-matched or crossmatched compatible platelets may offer satisfactory results. However, patients refractory to all available platelet concentrates may benefit from intravenous immunoglobulin (IVIg), plasma exchange, massive ABO-identical platelet transfusions, and acid-treated platelets (stripped of HLA antigens).

Platelets are stored at 20–22°C (room temperature) with agitation for no more than 5 d. More recently (April 2005), the Food and Drug Administration (FDA) extended the shelf-life of apheresis platelets to 7 d only if collected by the Trima® COBE Spectra™ blood separator in conjunction with 100% testing by bacterial culture system, bioMerieux BactT/ALERT Microbial Detection System Release Test®, to monitor for any bacterial contamination.

4.4. Granulocytes

Current blood separators can collect granulocytes at high yields (20 to 30×10^9 granulocytes) from donors stimulated with recombinant granulocyte colony-stimulating factor (G-CSF) and steroids; however, granulocyte transfusions lost popularity between 1985 and 1995 because of reported adverse pulmonary reactions and marginal clinical results. The unimpressive clinical efficacy may be attributed to rapid postcollection neutrophil apoptosis and inadequate doses (because previous donors did not undergo any stimulation/mobilization). Renewed interest in granulocyte transfusion stems from the availability of G-CSF that not only increases the yield of granulocyte collections but also inhibits neutrophil apoptosis. Currently, granulocyte transfusion has limited indications that include refractory fungal or bacterial infections in neutropenic patients and those with qualitative neutrophil defects. Granulocyte transfusions may also be beneficial to newborns with sepsis, neutrophil counts of less than 3000/μL or a defective marrow response. Granulocytes have no defined regulatory specifications because the FDA does not license them. However, the American Association of Blood Banks (AABB) requires that each unit contain at least 1.0×10^{10} granulocytes *(***Note:** value intended as a goal for adequate collection but not as an adequate clinical dose). At least four consecutive transfusions of 1.0×10^{10} granulocytes are recommended. The use of additional transfusions is based on the patient's response or clinical course and the clinician's judgment. Granulocyte units are suspended in 200 to 400 mL plasma and contain significant numbers of platelets (1 to 6×10^{11} platelets, equivalent to an apheresis platelet unit), RBCs, and viable lymphocytes. Thus, they must be ABO and Rh compatible with the recipient and irradiated to prevent transfusion-associated (TA)-GVHD.

Granulocytes have to be transfused as soon as possible and, if necessary, stored without agitation at 20–24°C for no more than 24 h.

4.5. Fresh-Frozen Plasma

Fresh-frozen plasma (FFP) is a single-donor unit of plasma that has been separated from one unit of whole blood or collected by apheresis and frozen at –18°C or lower within 6 to 8 h of collection. It contains coagulation factor levels at 1 U/mL (or 100% activity) and is indicated to treat global or multiple coagulation factor deficiencies (i.e., liver failure and dilutional coagulopathy in massively transfused patients). Other uses for FFP include emergent reversal of warfarin therapy (when time does not allow the use of vitamin K) and plasma exchanges in thrombotic thrombocytopenic purpura and hemolytic uremic syndrome. The timing and dose are important. Correction of a markedly abnormal prothrombin time and activated partial thromboplastin time requires FFP transfusions immediately before surgery because several of the coagulation factors have very short half-lives (particularly factor VII with a biological half-life of 3 to 6 h). The dose is based on the patient's weight at 10 to 20 mL/kg. FFP contains ABO antibodies and therefore must be compatible with the recipient's red cells.

4.6. Cryoprecipitated Antihemophilic Factor

Cryoprecipitated antihemophilic factor (CRYO) is the cold insoluble portion of plasma processed from FFP by cold precipitation. The precipitate is formed as FFP is thawed at 1 to 6°C. The stability of the coagulation factors is maintained for up to 1 yr at –18°C storage. It is a plasma-derived product that contains the highest concentration of fibrinogen (250 mg, primarily used in acquired hypofibrinogenemia of DIC), factor VIII (80–120 U/concentrate), and a variable percentage of the original plasma concentration of von Willebrand factor (40–70%) and factor XIII (30%). It is indicated for the treatment of hemorrhagic disorders resulting from either quantitative or qualitative defects of these factors. However, the availability of factor concentrates and recombinant factors have contributed to its diminished usage. CRYO is also used as a source of fibrinogen to form a fibrin glue or sealant (when added to thrombin) and is also indicated to ameliorate the platelet dysfunction in uremia. Like FFP, it contains ABO antibodies, requiring consideration of recipient red cell compatibility.

4.7. Immunoglobulins

Parenteral Igs are manufactured from pooled human plasma and are used in primary or acquired hypogammaglobulinemia to protect against viral and bacterial infections. They consist of all subclasses and allotypes of IgG with only trace amounts of IgM and IgA. Polyvalent/multispecific immunoglobulins offer

protection against many infections, whereas hyperimmune/specific immunoglobulins are obtained from actively immunized donors and contain high titers of disease-specific antibodies (i.e., hepatitis B virus [HBV]), *Varicella zoster*, rabies, or cell-specific [RhIg]). Rigorous donor screening and modified Cohn fractionation have made immunoglobulin preparations relatively safe.

Immunoglobulins can either be administered intravenously (IVIg) or intramuscularly (immune serum globulins) and are generally well tolerated. Adverse effects are rare (<1%) and include headache, nausea, vomiting, chills, volume overload, and allergic and pulmonary reactions that can be prevented by slow infusion and pretreating with diphenhydramine and/or hydrocortisone. True anaphylactic reactions are very rare and are seen in common variable immune deficiency and IgA deficiency. IgA deficient products are recommended in these cases.

Polyvalent immunoglobulins are indicated in hypogammaglobulinemias (congenital or acquired) and for immunomodulation in certain disorders. **Note:** the plasma half-life of IgG (the major human immune globulin) is 21–23 d, but longer in hypogammaglobulinemia. In hypogammaglobulinemia, a dose of 0.4–0.6 g/kg body weight is given every month to maintain serum IgG levels. In acquired hypogammaglobulinemia (e.g., multiple myeloma), immunoglobulins are recommended in patients with frequent infections. Established indications for immunoglobulin replacement or prophylaxis are parvovirus B19 infections (among immunosuppressed patients) and recurrent life-threatening infections (among HIV-infected children). The use of prophylactic IVIg in adults with advanced HIV infection has shown equivocal results.

High-dose polyvalent immunoglobulins achieve immunomodulatory effects through multiple mechanisms: (1) by suppressing antibody production directly through its effect on B-lymphocytes, (2) by interfering in the interaction between autoantibodies and cellular targets through its anti-idiotypic antibodies, and (3) by blocking the Fc-receptors on macrophages. Higher doses of IVIg (e.g., 0.4 g/kg × 5 d) are recommended for immunomodulation in Kawasaki syndrome (a mucocutaneous lymph node syndrome in children) and idiopathic thrombocytopenic purpura.

5. ADVERSE REACTIONS TO BLOOD TRANSFUSION

5.1. Acute Hemolytic Transfusion Reactions

Acute hemolytic transfusion reactions (AHTR) involves rapid destruction of blood cells immediately or within 24 h of a transfusion, commonly of RBCs or whole blood. It has a low rate of occurrence but is the most dangerous complication with a high mortality. Mortality depends on the amount of incompatible

blood infused (25 to 44 % with infusion of less than or more than 1000 mL, respectively). ABO incompatibility is the most common cause of immune AHTR and accounts for 74% of all fatal reactions. AHTR is due to preformed antibodies, often IgM (commonly anti-A), that bind complement and cause red cell lysis. Nonimmune causes of hemolysis mimic immune mediated hemolysis and have to be excluded. Chemical, mechanical, thermal, and physical damage of red cells may result from bacterial contamination, mechanical trauma during infusion, thermal hemolysis (from overheating or freezing a unit), and osmotic hemolysis (use of hypotonic reconstituting solutions and co-administration of drugs during transfusions)

The patients commonly experience fever and chills, hypotension, and chest pain. Other signs and symptoms include low back pain, flushing, dyspnea, hemoglobinuria, gastrointestinal symptoms (abdominal pain, diarrhea, and vomiting), and unexpected bleeding from DIC. In patients under anesthesia, no immediate reactions may be recognized. Changes in blood pressure, diffuse bleeding, and hemoglobinuria may be the only signs. AHTR may cause oliguria and acute renal failure.

The classic laboratory changes include a drop in hemoglobin and hematocrit, hemoglobinemia (free hemoglobin can be demonstrated in serum or plasma), hemoglobinuria, reduced serum haptoglobin, and positive DAT. Elevations of unconjugated bilirubin, methemalbumin, and lactate dehydrogenase, and an unexpected red cell antibody may be present.

The signs and symptoms of an IgM-type acute hemolysis result from complement-mediated lysis of red cells and the release of cytokines. Binding of complement to the red cell surface is a major factor in cytokine production. The symptoms of IgG-type hemolysis also involve several cytokines (IL-1, IL-6, IL-8, and TNF). Coagulopathy, frequently seen in hemolytic transfusion reactions, especially due to IgM antibodies, results from several mechanisms. The coagulation cascade is activated by (1) antigen–antibody complexes (activates Hageman factor); (2) thromboplastic substances of the red cell stroma; (3) platelet factor 3 released by activated platelets; and (4) tissue factor release secondary to hypotension.

In order to minimize AHTRs, all transfusions should have clearly defined indications. If an AHTR is suspected, the transfusion must be stopped and immediate steps taken to confirm or exclude this possibility. Management of AHTR is dependent on the clinical status of the patient and may include cardiopulmonary support, prevention of renal failure (i.e., fluid resuscitation, vasopressors, and diuretics), and treatment of DIC. Severe bleeding in DIC requires platelets, replacement of procoagulant factors and fibrinogen (FFP and CRYO transfu-

sions), and administration of low-dose heparin. Heparin limits both hemolysis (by its direct anticomplement activity) and inappropriate activation of procoagulant activity (by enhancing antithrombin III-neutralizing serine proteases).

5.2. Delayed Hemolytic Transfusion Reaction

Delayed hemolytic transfusion reaction (DHTR) represents an anamnestic response to a red cell antigen to which the patient has been previously sensitized by pregnancy or previous transfusions. It occurs in patients without identifiable antibody detected in the pretransfusion compatibility testing who experience accelerated red cell destruction of the transfused red cells after an interval of 3 to 10 d from transfusion. Such reactions are due to weaker antigen–antibody reactions and may develop over days. Because of the low titer of reactivity, the implicated antibody is not detectable at the time of screening or compatibility testing. The sensitivity of the antibody-screening test is important in preventing DHTRs because insensitive tests will miss weak-reacting antibodies. **Note:** these weak-reacting antibodies commonly include Rh antibodies (against CEce) and other antibodies in the Kell (anti-K), Kidd (anti-JK^a), and Duffy (anti-Fy^a) blood group systems.

DHTRs often remain asymptomatic and have milder symptoms than AHTRs, including unexplained anemia, jaundice, and fever. In patients with sickle cell disease, DHTRs may precipitate a sickle cell crisis. Laboratory findings are similar to those of AHTR, except for the identification of a new alloantibody in the patient's RBC eluate or serum or both. The degree of hyperbilirubinemia will depend on the rate and amount of hemolysis and the patient's liver function. Hemolysis is usually extravascular but intravascular hemolysis can occur. Both AHTRs and DHTRs can demonstrate a positive, mixed-field, or negative DAT. A mixed-field reaction will show a mixture of agglutinated transfused donor cells along with unagglutinated patient's cells. The DAT can be negative if all the incompatible transfused donor cells are immediately destroyed.

There is a need to differentiate DHTR from delayed serological transfusion reactions (DSTRs), in which only serological incompatibility is evident without clinical evidence of hemolysis. DSTRs are more common than DHTRs in the multiply transfused patient as more sensitive screening methods are employed and the length of stay for in-patients increases.

DHTRs are tolerated well by many patients and may only require close monitoring. Typically, fluid loading and diuresis are not indicated unless active intravascular hemolysis is present. Complications, such as renal failure and sickle cell crisis, should be treated accordingly. A red cell exchange is indicated if there is a large burden of antigen-positive cells. IVIg may be useful because extravascular hemolysis is similar to acute immune hemolytic anemia. Transfusion should

be avoided until the causative antibody is identified and antigen-negative units are available. Withholding transfusions because of the lack of serologically compatible blood in patients with severe anemia is associated with significant morbidity. This can be avoided by good communication between the clinician and transfusion service.

5.3. Febrile Nonhemolytic Transfusion Reactions

Febrile nonhemolytic transfusion reactions (FNHTR) are defined as a temperature rise of at least 1°C in association with a transfusion or up to 4 h after that may be accompanied by chills or rigors. Such reactions are due to acquired antibodies to donor leukocyte antigens or pyrogenic cytokines (IL-1, IL-6, IL-8, and TNF-α) elaborated by leukocytes present in the blood components or products. FNHTRs are less frequent with prestorage leukocyte-depleted blood components. They occur in 0.5–1.4% of all transfusions. The fever is self-limited and resolves after 4–6 h. Antipyretics are effective as symptomatic treatment. Meperidine can be useful for treating rigors. Among patients who have experienced a FNHTR, there is a 15% recurrence rate.

5.4. Allergic Reactions

Allergic reactions are probably the most frequent, occurring in 1–2% of all transfusion reactions. The symptoms range from local or diffuse pruritus, urticaria, erythema, and cutaneous flushing to anaphylactic allergic reactions occurring within minutes of the transfusion. Anaphylactoid reactions fall in between the two ends of the spectrum.

Allergic disorders afflict 30% of donors, and passive transfer of IgE antibodies may be involved. Uncomplicated allergic reactions are associated with increased histamine (increased during storage), cytokines, mast cell activators (i.e., leukotrienes), and other vasoactive substances ($C3_a$ and $C5_a$) produced by donor leukocytes during storage. Some allergic reactions only have pulmonary signs and symptoms without cutaneous involvement (10%).

Severe anaphylactic reactions may occur after infusion of a very small volume (<10 mL) in patients with IgA deficiency and are due to preformed, class specific, recipient anti-IgA antibodies against infused donor IgA proteins. Additionally, they may be due to antibodies against complement C_4 and haptoglobin. Patients with Chido (Ch) and Rogers (Rg) antibodies (against the Ch/Rg blood group antigens carried by complement C4d of the classic complement pathway) also exhibit anaphylactoid reactions following plasma product transfusions. Haptoglobin deficiency is rare in North American populations but more common than IgA deficiency among Japanese patients experiencing anaphylactic reactions.

Anaphylactoid reactions are typically associated with subclass, allotypic, or specific anti-IgA in patients with normal or demonstrable levels of IgA. These

reactions may be seen in other transfused products, such as peanut allergen transfused to patients with peanut allergy.

Mild uncomplicated allergic reactions involving hives (without other symptoms) respond to antihistamines (such as diphenhydramine). The transfusion may be restarted after treatment if there is no recurrence or progression of symptoms. Restarting a transfusion is not advisable with more serious reactions; in particular, pulmonary symptoms with airway involvement. Treatment of severe anaphylactic reactions is the same as for any anaphylactic reaction and requires immediate cessation of the transfusion, epinephrine, and other supportive care. Prevention of anaphylaxis in IgA-deficient individuals requires avoidance of all plasma containing products unless collected from a known IgA-deficient donor and washing of all red cell and platelet products.

6. DISEASES TRANSMITTED BY BLOOD TRANSFUSION

The major concern is to make all blood transfusions as safe and effective as possible. Several infectious agents, viruses, bacteria, and parasites are transmissible by transfusion of whole blood, blood components, or products. Attention will focus on those transfusion-transmissible diseases (TTD) that present risks of chronic infection in the recipient.

Major strategies available to reduce transmission include: stringent donor selection and laboratory testing; use of autologous blood, pharmacological substitutes, or new transfusion strategies; inactivation of residual infectious agents in the units to be transfused, and limiting the number of donor exposures and allogeneic transfusions.

Blood donor screening is one of the more important steps in protecting the safety of the blood supply. Nonremunerated, voluntary donors with low infectious risks are encouraged and recruited to become blood donors. The scarcity of the blood supply in many countries has spurred the use of paid donors. Direct interviews, miniphysical examinations and laboratory testing of all blood collections help to exclude donors who have exposure to transmissible infections or who have the risk factors for TTD.

The blood supply is screened or tested for bacterial and viral infections (including tick borne infections), parasites (i.e., malaria, babesia, Chagas' disease, and microfilariasis).

Blood intended for autologous (only if transfused outside the collection facility) and allogeneic use require tests for syphilis (STS), HBV (HBV surface antigen [HBsAg] and antibodies to the hepatitis core antigen [anti-HBc]), hepatitis C virus (HCV, anti-HCV, and HCV RNA), HIV (antibodies to HIV-1/HIV-2 and HIV-1 RNA), and human T-cell lymphotropic virus (anti-HTLV-I and II). Other infectious agents can be tested or excluded based on the epidemiology of the infectious agent, geographic area, and intended use of the blood product. In

Table 3
Risk Estimates of Transfusion-Transmitted Infections in the United States[a]

Infection	*Risk estimate (per unit transfused)*
Hepatitis B	1/205,000
Hepatitis C	1/1,935,000
HTLV	1/2,993,000
HIV	1/2,135,000

[a]Estimates of residual risk in donations from repeat donors of the American Red Cross blood donor population from 1995 to 2001 (after NAT testing for HIV and HCV).

From Dodd RY, Notari EP 4th, Stramer SL.Current prevalence and incidence of infectious disease markers and estimated window-period risk in the American Red Cross blood donor population. *Transfusion* 2002;42(8):975–979.

countries with endemic malaria, testing for malaria parasites is mandatory. The general safety of the blood supply will be enhanced if these concerns are addressed; however, minimal risks still remain because very recent infections are not detected by recommended/standard laboratory tests (*see* Table 3).

6.1. Bacterial Infections Transmitted by Blood Transfusion

Bacterial contamination resulting in transfusion-associated bacterial sepsis is now believed to be the most common infectious source of morbidity and the most frequently reported cause of transfusion-related fatalities in the United States (accounting for 16% of transfusion fatalities). Bacteria are believed to originate from the donor either from the venipuncture site or unsuspected bacteremia. Excluding donors with chronic diseases or recent febrile infections and scrupulous disinfection of the skin reduces the risk of bacterial transmission. Both Gram-positive and -negative bacteria have been implicated.

Bacterial multiplication is more likely in components stored at room temperature (i.e., platelets) than refrigerated components (i.e., RBCs) or frozen components (FFP and CRYO). Bacterial contamination of platelet transfusion is one of the most common infectious risks of transfusion, causing life-threatening sepsis in 1 in 100,000 recipients and immediate fatal outcome in 1 in 500,000 recipients. Despite limiting platelet storage to 5 d, various pathogens have been implicated (*Staphylococcus*, *Enterobacteriaceae*, *Streptococcus*, *Bacillus*, and *Pseudomonas*).

The low incidence of platelet-associated sepsis may be due to underreporting because (1) they typically occur several hours after transfusion and are not as catastrophic as RBC-associated sepsis; and (2) sepsis is attributed to other causes because platelets are commonly transfused to immunocompromised patients with other complex problems.

Psychrophilic Gram-negative bacteria can multiply in refrigerated blood and components. RBC contamination is primarily from *Yersinia enterocolitica* and *Serratia liquifaciens*. *Y. enterocolitica* proliferates and produces an endotoxin in refrigerated anticoagulated blood because it can grow at temperatures below 37°C in a calcium-free medium even after a long lag period. Any bacterial contamination of blood products is potentially serious.

P. cepacia and *P. aeruginosa* are environmental organisms that grow optimally at 30°C and have been isolated from cryoprecipitate and plasma thawed in contaminated water baths.

Syphilis transmission by blood transfusion is possible but its occurrence is extremely rare because the phase of spirochetemia is short and the infective organism, *Treponema pallidum*, does not survive refrigerated storage for more than 96–120 h. Seroconversion occurs after the phase of spirochetemia so testing donor blood by standard STS does not effectively prevent transmission. However, most positive STS results are demonstrated by donors with inadequately treated noninfectious syphilis, or are biological false positives (may be positive for hepatitis, mononucleosis, measles, chickenpox, immunizations, rheumatoid arthritis, and pregnancy). There have only been two cases of transfusion-transmitted syphilis reported in the English literature. Another spirochete, *Borrelia burgdorferi*, causing Lyme disease (transmitted by the Ixodes deer tick) can survive routine storage of RBCs and FFP. However, transfusion-related transmission has not been reported at this time. The phase of spirochetemia is associated with clinical symptoms that would render potential donors ineligible for donation. Donors diagnosed with Lyme disease are accepted provided they have completed antibiotic therapy and are completely asymptomatic.

6.2. Viral Diseases Transmitted by Blood Transfusion

6.2.1. Hepatitis

Before the 1980s, the transmission of hepatitis was the major transfusion-related viral infection. The absolute number of hepatitis infections post-transfusion has decreased significantly, because reliable tests for HBV and HCV were introduced. In the past, donors having elevated alanine aminotransferases were rejected. This surrogate marker is no longer used with the availability of more specific tests for anti-HCV and HCV RNA. Anti-HBc testing has been retained

because it may still detect a few donors with infectious HBV who are negative for HBsAg.

Hepatitis A (HAV), an RNA virus, is generally transmitted by the oral-fecal route and very rarely transmitted by transfusion. The main concern of HAV (and parvovirus B19 as well) is transmission by plasma derivatives (particularly human source factor VIII concentrates) because it does not have a lipid envelope and is not inactivated during the manufacturing process. Nucleic acid testing (NAT) is available and is done only for *source plasma* (plasma intended for manufacture of blood derivatives/products). NAT testing for this virus is currently considered an "in-process control" by the FDA so donor notification (for positive tests) is not required. In contrast to persons infected with HBV, persons who are exposed to HAV do not develop a chronic carrier state. Blood donors are not routinely screened for HAV because of the rarity of transfusion transmissible HAV and the absence of HAV antibody at the time of viremia. The risk for HAV is estimated at 1 per 10 million units.

HBV, a DNA virus, is primarily transmitted by the parenteral route. It can be transmitted within the first 100 d after infection when viremia is present and no protective immunity has yet developed, or in the chronic carrier state. Donor screening to detect HBV infection includes assays for the HBsAg and for anti-HBc. Most infections are asymptomatic and HBsAg positivity occurs 2 to 6 wk before the onset of symptoms; thus, an apparently healthy but infectious donor will be eligible for donation.

A "window period" for any transfusion-transmissible agent is defined as the period of time that an individual is potentially infectious but demonstrates negative serological tests (without detectable antibodies). A licensed NAT test is not available for HBV because of low levels of viremia during the "window phase" resulting from a slow viral doubling time. However, a recent publication reported that the "window period" can be reduced by 25–36 d using single donor NAT (SDNAT), further reduced by 9–11 d using minipool NAT (MPNAT) and reduced by 2–9 d using a new and more sensitive HBsAg assay. Disagreement exists as to whether HBV-NAT will be cost effective to further reduce the risk of transfusion-transmissible HBV.

HCV, an RNA virus, was discovered in 1989 and was linked with most cases of non-A, non-B hepatitis in the past. HCV infections are mild and generally (80%) asymptomatic. The long-term effects are far more serious in that the majority of HCV infected patients develop chronic liver disease with 20% developing cirrhosis. In addition, those with HCV cirrhosis develop hepatocellular carcinoma. It was estimated that the elimination of hepatitis C by anti-HCV antibody testing prevented 40,000–50,000 new cases of post-transfusion hepatitis per year (an additional 10,000–13,000 cases may have been prevented by

newer versions of the test). The risks of transfusion-transmissible HCV has further been reduced by HCV-NAT. Prior to HCV-NAT, the window period because of a slower doubling time was 70 d. The newly developed HCV-NAT (MPNAT/SDNAT) has further closed the window phase to 10 d, so that the transfusion-transmissible risk for HCV is reduced to 1:1,935,000 per unit transfused from 1:276,000 in the past (*see* Table 3).

Hepatitis D (or δ), another RNA virus, exists as a co-infection in patients chronically infected with HBV because the "delta agent" cannot multiply in the absence of HBV. Testing for HBV markers eliminates hepatitis D-positive donors.

The hepatitis G virus (HGV, also known as GBV-C), a newly discovered RNA virus, is potentially transmitted by blood products. Among normal donors, the reactivity is 1–4% by PCR techniques. Currently, HGV has not been associated with a specific disease entity and its designation as a "hepatitis" virus may have been premature. Thus, no routine donor testing is performed.

Two other hepatitis viruses, hepatitis E virus (HEV) and SEN virus, are apparently transmitted by transfusion. HEV does not occur in the United States but is endemic in other parts of the world, although its true incidence remains unknown. SEN virus is the latest virus postulated to cause the remaining non A–E transfusion-transmitted hepatitis. At present, the biological role of SEN virus has not been clearly defined.

Lastly, TT virus, a DNA virus, first described by a Japanese group was originally postulated to cause non-A–E hepatitis. This virus is prevalent in many countries and is currently not associated with hepatitis.

6.2.2. Retroviruses

Retroviral infections transmitted by blood transfusion include HIV-1 and HIV-2 and human lymphotropic virus (HTLV-I and HTLV-II). HIV is transmitted by both cellular blood components and plasma; however, both types of HTLV are highly cell-associated and require viable lymphocytes for transfusion transmission.

HIV is a cytopathic retrovirus that preferentially infects CD4-positive T-lymphocytes. The infection begins as a viremia of cell-free virions that may be clinically manifested by an acute nonspecific flu-like illness. Viremia is detectable in the plasma 10 d to 3 wk after infection. As HIV antibodies appear, the disease enters a clinically latent phase. Viral replication and dissemination continues and the virus can be transmitted by blood or genital secretions.

HIV-2 causes endemic infection in West Africa and has apparently spread with population movements. It is indistinguishable from HIV-1 in disease spectrum although HIV-2 has a longer incubation period and is less efficiently transmitted than HIV-1.

The AIDS epidemic in the early 1980s had a catastrophic impact on the safety of blood transfusions but triggered a major impetus for improvement in blood safety. The risk of HIV transmission through blood components/products dramatically decreased with more stringent donor history screening and improvement of donor testing (*see* Table 3). Combination HIV-1/HIV-2 tests implemented in the United States in 1992 have identified to date three HIV-2 infected donors, none appear to have been infected in the United States.

Prior to 1992, the window period for HIV averaged 45 d. More sensitive HIV-antibody screening tests closed this window to 22 d. In 1996, HIV antigen (p24 antigen) testing reduced this infectious window by an estimated 6 d; such that circulating cell-free virions could be detected as early as 16 d following infection. With the newly developed PCR-based NAT, this window period was further reduced to about 10 d. Although SDNAT has a detection sensitivity of less than 50 viral copies/mL, its higher cost prompted many blood centers into using MPNAT with 14–16 donors per pool. An estimate of residual risk of HIV infection from repeat donors after NAT is 1 per 2,135,000 (per unit transfused). Currently, many blood centers have implemented NAT and discontinued p24 antigen testing following the FDA's licensure of the HIV-NAT assay.

HTLV-I and II are human retroviruses that can be transmitted by blood, sexual contact (predominantly male-to-female), and through breast milk. It circulates as a provirus that is incorporated into the DNA of lymphocytes. No cases of transfusion-transmitted HTLV have been reported with noncellular blood components. Prolonged storage for more than 10 to 14 d (refrigeration inactivates the lymphocytes) also reduces transmission risk. Compared to other viruses, HTLV-I and -II transfusion transmission is less efficient because exposure invariably does not result in infection. Look-back studies have shown that one in three HTLV-contaminated units transmitted the virus.

Risk factors for HTLV-1 infection are birth or sexual contact in areas where the disease is endemic (Japan and certain Pacific Islands, Caribbean basin, sub-Saharan Africa, Central and South America). The natural epidemiology of HTLV-II is not fully known, although a high prevalence is seen in some Native American populations. HTLV-II is presumably associated with parenteral transmission with risk factors of intravenous drug use (1 to 20% seroprevalence) and sexual contact with an IV drug user.

An excess of infectious syndromes (i.e., bronchitis, pneumonia, and urinary infections) is seen among blood donors infected with HTLV-I or -II. However, most HTLV-1-positive individuals remain asymptomatic donors with an extremely long latency period (decades) and a 2–5% life-long risk of developing adult T-cell lymphoma/leukemia or HTLV-associated myelopathy/tropical spastic paresis in areas of high endemicity. Enzyme immunoassay (EIA) for both HTLV-I and -II is used to screen donors, and EIA-reactive donors are indefinitely

deferred regardless of investigational supplemental tests since there is no licensed confirmatory test.

The risk of transfusion-transmissible HTLV with the same "51-d window period" has decreased from 1:514,000 (in 1998–1999) to 1:2,993,000 (in 2000–2001). Such a dramatic risk reduction may be partly attributed to the implementation of universal leukocyte reduction. Viral load reduction by removal of infected leukocytes remains controversial.

6.2.3. Human Herpesviruses

Leukocytotropic human herpesvirus (HHV) may contaminate blood components. Cytomegalovirus (CMV or HHV-5) and Epstein-Barr virus (EBV or HHV-4) have the greatest clinical relevance to transfusion medicine.

CMV is transmitted by transfusion in the latent, noninfectious state in the genome of leukocytes present in cellular blood components. Seropositivity in the general population, United States ranges from 20 to 80%, but only a small fraction of these individuals have circulating virion. Exposure and host factors determine symptomatology. Among immunocompetent individuals, CMV causes a mononucleosis-like syndrome or an asymptomatic infection and remains latent in tissues and leukocytes.

Symptomatic CMV infections develop in immunosuppressed, seronegative hosts. Human progenitor cell transplant (HPCT) recipients (develop CMV pneumonitis), low birth weight neonates (<1500 g) of seronegative/seropositive mothers and HIV-infected patients (develop CMV chorioretinitis, encephalitis, and enteritis) are particularly at risk. Among seropositive HPCT recipients, viral reactivation is the most common cause of CMV infection (up to 69% in one study). Generally, the donor organ is the source among transplant recipients. Additionally, the risk of transfusion-transmitted CMV is high in heavily transfused recipients (liver, heart-lung, and pancreas transplants).

"CMV-reduced-risk blood components" are recommended to reduce CMV transmission. Leukoreduced cellular blood components are comparable to seronegative cellular blood components. Nonetheless, there remains a small risk of transmission with either type of component. Most centers in the United States, Canada, Australia, and Europe recommend transfusion of CMV-negative blood components to CMV-negative pregnant women and for intrauterine transfusions (to prevent transplacental infection), and to CMV-negative immunosuppressed individuals (i.e., HPCT recipients, HIV-positive with AIDS, and other immunosuppressed individuals). It is also prudent to extend this consideration to CMV-negative candidates for HPCT. Despite transfusions of "CMV-reduced-risk blood components," a few marrow transplant patients (1 to 4%) still develop primary CMV infection.

EBV targets B-lymphocytes causing polyclonal proliferation with T-lymphocyte response and demonstration of "atypical lymphocytes." It causes infectious

mononucleosis, the endemic form of Burkitt's lymphoma in Africa and nasopharyngeal carcinoma. EBV can be transmitted by blood transfusion, but is a rare cause of significant disease in immunocompetent individuals. Transfusion-transmitted EBV is usually asymptomatic. Rarely, EBV causes posttransfusion hepatitis and "postperfusion syndrome." The latter is characterized as a viral-like illness following massive transfusion of fresh blood during cardiac surgery.

EBV contributes to the development of lymphoproliferative disorders among immunosuppressed HPCT and organ transplant recipients from a reactivation of a latent infection. The high seropositivity rate (90%) among blood donors and the low risk of acquiring clinical disease among immunocompetent recipients make blood donor screening and laboratory testing less beneficial.

6.2.4. Other Viruses and Unconventional Infectious Agents (Prions)

Parvovirus B19 is the etiological agent of erythema infectiosum ("fifth disease") in children, arthritis in adults, and hypoplastic anemias in HIV-infected individuals. More ominously, it causes an aplastic crisis among patients with chronic hemolytic anemias who rely on active erythropoiesis to offset the shortened red cell survival. The red cell P antigen is the cellular receptor for parvovirus B19 so those who do not possess the antigen are naturally resistant to infection. The presence of parvovirus B19 antibodies (30 to 60% prevalence) and the brief viremia in blood donors make viral transmission uncommon (ranging from 1 in 3300 to 1 in 50,000). The rarity of clinically significant disease or viral transmission has not made donor screening and testing imperative. There are reports of parvovirus B19 transmission by solvent-detergent plasma (solvent detergent viral inactivation is ineffective because the virus lacks a lipid envelop), cellular blood components, and clotting factor concentrates. There are no reports of transmission from IVIg and albumin. NAT screening has been implemented only for in-process manufacturing control of plasma derivatives.

West Nile virus (WNV), a flavivirus, is transmitted through mosquito bites (an arthropod-borne-virus), blood transfusions (first reported during the 2002 epidemic), and organ transplants to humans causing encephalitis, meningitis, and very rarely asymmetrical flaccid paralysis. Immunocompromised and elderly individuals are at risk of developing severe disease. Viremia occurs 1 to 3 d following an infecting mosquito bite and lasts from 1 to 11 d.

WNV transfusion-transmission risk (per 10,000 donations) ranges from 1.46 to 12.33 for selected metropolitan areas and from 2.12 to 4.76 for six high-incidence states. Serological tests are ineffective in donor screening as viremia disappears by the time IgM antibodies are detected by ELISA tests. The seasonal increase in 2003 prompted MPNAT under a clinical protocol in the United States. Likewise, donor history questionnaires have been implemented to reduce the risk of WNV transmission. During periods of high endemicity, frozen products have

been withdrawn voluntarily from the supply, as cessation of donor collection is not feasible. Other blood derivatives do not appear to be at risk for transmission as the WNV is inactivated by heat or solvent-detergent treatment.

The severe acute respiratory syndrome (SARS) virus that first appeared in Guangdong, China is spread by close person-to-person contact and is believed caused by a *corona virus* that causes the common cold and/or probably a *paramyxovirus*. Its spread to other countries was linked to airline travel, often by health care workers in contact with SARS patients. The virus has been isolated from the blood of an infected individual but its risk of transmission through blood transfusion remains unknown. However, because of its highly contagious nature, transfusion transmission is possible if blood collection coincides with the viremic phase of the disease. The FDA recommends deferral of at-risk donors for 14 d after a possible exposure and at least a 28-d deferral after resolution of symptoms. Likewise, deferral is extended to donors with a history of travel or residence in SARS-affected areas.

Transmissible spongiform encephalopathies (TSEs) are rare, fatal degenerative neurological disorders caused by infectious agents classified as prions or proteinaceous infectious particles that lack nucleic acid. A prion is an abnormal isoform (PrP^{SC}) of a normal cellular protein (PrP^{C}) that is resistant to inactivation by alcohol, formalin, ionizing radiation, proteases, and nucleases; but is disrupted by autoclaving, phenols, detergents, and extremes in pH. TSEs have long incubation periods (years to decades).

Two such TSEs, classic Creutzfeld-Jakob disease (CJD) and variant Creutzfeld-Jakob disease (vCJD) are important from the transfusion medicine perspective. Unlike classic CJD, which presents in older patients, vCJD is observed in young adults and has an acute course with rapid progression to death in 2 yr.

The majority of classic CJD is sporadic (80%). Familial cases (10–15%) are caused by mutations and the rest (10%) arise from iatrogenic transmission (administration of growth hormone and gonadotropic hormone derived from pooled human pituitary tissue, allografts of dura mater and cornea, and reuse of intracerebral electroencephalographic electrodes from such patients). To date, transfusion transmission of CJD has been reported in experimental rodent models, but not in humans. Although theoretically possible, there is growing consensus that CJD transmission by blood or its components is unlikely.

Variant CJD (vCJD), first reported in the United Kingdom in 1996, is caused by the same prion responsible for bovine spongiform encephalopathy (BSE) but might be entirely different. Its potential for transmission by blood and blood components is heightened by the fact that vCJD can spread from cattle to humans (presumably by ingestion) and from human to humans (a recipient developed

symptoms 6.5 yr after a red cell transfusion). In December of 2003, a potential case of vCJD associated with blood transfusion was reported in the United Kingdom. Its presence in lymphoreticular tissue of vCJD patients, determined by animal studies and its association with B-lymphocytes, suggests possible transmission through blood transfusions. As a safety measure, several European countries and Canada implemented universal leukocyte reduction for all blood products to prevent lymphocytes from transmitting vCJD. However, the efficacy of such intervention remains uncertain.

Expanded donor deferral criteria have been implemented to reduce the risk of transmission of both TSEs. This includes deferral of donors at risk of exposure due to travel or residency in areas with BSE epidemics, deferral of donors who received bovine insulin and pituitary-derived human growth hormone or dura transplants since 1980, and deferral of donors with blood relatives diagnosed with CJD.

6.3. Parasites Transmitted by Blood Transfusion

Malaria is caused by several species of the intraerythrocytic protozoan *Plasmodium* and can be transmitted by transfusion of parasitemic blood. Malarial parasites survive for at least 1 wk in blood components stored at room temperature (i.e., platelets) or at 4°C. They can survive cryopreservation with glycerol. Any blood component that contains red cells can transmit infection via the asexual form of the parasite. It is frequently transmitted by red cell transfusions, rarely by platelet transfusions, and is absent in plasma products. It is recognized as a global health problem, but is very rare in the United States. However, it is the most commonly recognized parasitic complication of transfusion and occurs as at an estimated rate of 0.25 cases per 1 million blood units collected. Asymptomatic carriers are the general source of transfusion acquired infections. At present, there are no practical serological tests to screen asymptomatic carriers. Prevention of malaria transmission is possible by deferral of prospective donors with increased risk of infectivity based on medical and travel history. In Western Europe and the United States, blood donors are deferred for 12 mo after travel to malaria-endemic areas. Individuals born in areas endemic for malaria generally are excluded from blood donation for 3 yr after leaving the area.

Chagas' disease (American Trypanosomiasis) endemic in South and Central America is caused by another protozoan parasite, *Trypanosoma cruzi,* transmitted by reduviid bugs (cone-nosed or "kissing" bugs). Infection occurs from fecal contamination of the reduviid bug bite wound by the infectious trypomastigote. Acute infections are mild to asymptomatic and 20–40% of infected individuals enter a chronic phase of intermittent parasitemia manifested by "megasyndromes" (cardiomegaly, megaesophagus, and megacolon). Blood transfusion is a major

source of infection in South America, where parasite reduction using chemicals (like gentian violet) on donated blood pose additional risks. Six cases of transfusion-transmitted Chagas' disease have been reported in the United States (New York, Los Angeles, Texas, and Florida) and Canada, involving platelet concentrates in at least four cases. Currently, there is a 0.1 to 0.2% seroprevalence in areas with high immigrant populations from Central and South America. To date, no tests are licensed by the FDA for screening, but highly specific confirmatory tests (i.e., Western blot assays) are available. Close monitoring is needed to define the risk of transfusion transmitted Chagas' disease.

7. OTHER ADVERSE EFFECTS OF BLOOD TRANSFUSION

7.1. Iron Overload

Patients who are transfusion-dependent for aplastic anemias or chronic hemolytic anemias (sickle cell anemia and thalassemias) such as genetic hemochromatosis may develop iron overload (hemosiderosis), which may result in organ failure, primarily of the heart, liver, and pancreas. Every milliliter of transfused RBCs contains approx 1 mg of elemental iron. Signs of clinical toxicity become evident when total body iron reaches 400 to 1000 mg/kg of body weight. Patients who develop iron overload on chronic transfusion are candidates for chelation therapy with parenteral deferoxamine or oral iron chelators (*see* Chapter 23). Transfusion of RBC units enriched with a younger cell population or "neocytes" (reticulocytes), advocated by some investigators, potentially increases transfusion intervals and intravascular survival of RBCs (41%). However, its value in reducing hemosiderosis has not yet been established.

7.2. Transfusion-Associated GVHD

A transfusion recipient's ability to mount an immune response and inability to reject transfused (donor) T-lymphocytes in cellular blood components is fundamental to the pathogenesis of TA-GVHD. Only whole blood and its cellular components (RBCs, granulocytes, and platelets) containing sufficient, viable, cytotoxic T-lymphocytes can meditate TA-GVHD. No cases have been reported following FFP transfusions. This disease has been reported in immunosuppressed patients with hematological and solid malignancies. Rarely, it occurs after organ and HPCT and in patients receiving myeloablative therapy. Immunocompetent transfusion recipients at risk of TA-GVHD share a haplotype with related or unrelated HLA homozygous donors (HLA haploidentical). TA-GVHD typically appears 2 to 50 d after transfusion. The development of marrow aplasia and progressive pancytopenia distinguishes TA-GVHD from GVHD following

HPCT. These patients may develop a skin rash, diarrhea, fever, and abnormal liver function accompanied by extensive hepatocellular damage.

Most cases (>90%) of TA-GVHD are fatal. Treatment with immunosuppressive regimens (steroids, cytoxan, and antithymocyte globulin, including OKT3) has been ineffective. As a consequence, immunosuppressed patients (with congenital immunodeficiency syndromes, hematological malignancies undergoing myeloablative therapy, allogeneic or autologous HPCT) premature neonates (<1200 g), intrauterine transfusions, recipients of blood from biological relatives and HLA-matched platelets should receive only irradiated blood. Solid organ transplant recipients receiving immunosuppressive therapy or those undergoing chemotherapy/radiation therapy for solid tumors do not require irradiated blood componenets. Interestingly, TA-GVHD has not been reported in patients with AIDS. Gamma irradiation with 25–30 gy inactivates all immunoreactive lymphoid cells and prevents TA-GVHD. Leukocyte reduction is insufficient to prevent TA-GVHD.

7.3. Transfusion-Related Acute Lung Injury

The transfusion-related acute lung injury (TRALI) reaction is rare (1:2000 to 1:5000 units) and occurs during or within the 4–6 h after a transfusion. Patients suddenly develop shortness of breath with severe hypoxemia, chills, fever, cough, and tachycardia. Noncardiogenic pulmonary edema recognized on chest X-ray as bilateral diffuse interstitial infiltrates results from leukocytic activation and aggregation in the pulmonary bed causing "capillary leak." The pathophysiology of this reaction is thought to be immune-mediated, typically resulting from (1) donor antibodies (granulocytic or lymphocytotoxic antibodies) directed against white blood cell antigens, (2) HLA Class I and II antibodies, and (3) lipid activators of neutrophils in donor plasma. In very rare cases, these antibodies may be present in the recipient.

Most donors associated with TRALI are multiparous females or donors with multiple exposures to varying HLA types. Treatment is supportive; however, ventilatory and pressor support may be necessary. Resolution occurs within 3 to 7 d in 81% of cases but is fatal in a small proportion of cases. TRALI is the third leading cause of transfusion-related deaths, accounting for 13% of all transfusion fatalities.

8. AUTOLOGOUS BLOOD TRANSFUSION

An autologous donor donates blood for his or her own future use. For practical reasons, the transfusion of autologous RBCs is an attractive option for patients undergoing elective surgery. Autologous blood can be collected from a patient in advance of anticipated need (preoperative collection) or at the start of surgery

(acute normovolemic hemodilution); additionally, shed blood can be recovered for reinfusion during surgery (intraoperative collection) or during the postoperative period from drainage devices (postoperative collection). Clinicians should be aware that recovered blood does not provide platelets or coagulation factors.

Autologous blood transfusion avoids the small risk of transfusion-transmitted infectious agents, red cell alloimmunization, adverse reactions resulting from antibody-mediated hemolysis, and leukocyte-associated febrile reactions. In a larger perspective, autologous transfusion supplements and preserves the blood supply for other patients who acutely or chronically need allogeneic transfusions.

Autologous donors undergoing preoperative collection should be in satisfactory or good health without major cardiac problems or anemia. The predonation hemoglobin requirement is lower (11 g/dL) for autologous donation than for allogeneic donation (12.5 g/dL). Autologous donors can donate as often as every 72 h before the scheduled surgery. Ideally, supplemental iron is prescribed before the first collection because iron-restricted erythropoiesis is one of the limiting factors in collecting multiple units of blood over a short period of time. Oral or parenteral iron supplement enhances recovery of hematopoiesis. Rarely, recombinant human erythropoietin must be administered to donors who have insufficient erythropoietic response.

In order to justify the cost effectiveness of autologous collections, there should be a high likelihood that at least two units of blood will be used during surgery. Hospitals must establish guidelines regarding indications for autologous blood. During a 4–5 wk collection period, 2–4 units of RBCs can be collected. The storage time of autologous blood is comparable to blood from other donors (up to 42 d with additive solutions). Unused autologous blood may be discarded or may be used for allogeneic transfusions in facilities that allow "crossover" only after infectious disease testing. Only 30% of collections are typically eligible for allogeneic use. Many institutions choose not to "crossover" autologous units because of the cost, complexity, and error risks associated with the process. Freezing and long-term storage of RBCs is indicated for patients with rare blood group antibodies, making it difficult to find compatible blood (*see* p.439).

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