Developments in Component Therapy: Novel Components and New Uses for Familiar Preparations

MICHELLE S WRIGHT-KANUTH, LINDA A SMITH,

Over the years, the significant role of blood components in treating certain diseases or conditions has been recognized. The use of these components has expanded as patients undergo chemotherapy for bone marrow ablation and require short-term component support. On the other hand, these transfusions can cause reactions ranging from mild to severe. Despite advances in serological testing for infectious disease agents, the risk of infectious complications from transfusion still remains. In addition, newly identified agents that may be transmitted via transfusion are constantly identified.

The cellular components most people are familiar with include packed red blood cells (PRBC), washed PRBC, leukoreduced PRBC, and pooled or apheresis platelets. Plasma products such as fresh frozen plasma (FFP) or crytoprecipitated anti-hemophiliac factor (CRYO), on the other hand, may not be as familiar. As our understanding of how the immune system functions and as technology has progressed, specialized components or manufactured products such as blood substitutes have been advanced as remedies to some of the complications with component transfusion or to meet the ever-increasing need for these products.

In this article we will focus on some of the new uses of common components and uncommonly used or newly developing components. We will discuss their origins, composition, and the conditions or diseases they are used to treat. These components include:

- donor leukocyte infusions
- dendritic cell vaccines
- blood substitutes
- novel platelet products and substitutes
- intravenous immunoglobulin (IVIG)
- fresh frozen plasma and cryosupernatant in therapeutic plasma exchange.

The variety of products and conditions reflect the ever-expanding role of immunohematology in the treatment of disease.

The Focus section seeks to publish relevant and timely continuing education for clinical laboratory practitioners. Section editors, topics, and authors are selected in advance to cover current areas of interest in each discipline. Readers can obtain continuing education credit (CE) through P.A.C.E.® by completing the tearout form/examination questions included in each issue of CLS and mailing it with the appropriate fee to the address designated on the form. Suggestions for future Focus topics and authors, and manuscripts appropriate for CE credit are encouraged. Direct all inquiries to Carol McCoy PhD, CLS Continuing Education Editor, Department of Clinical Sciences, 343 Cowley Hall, University of Wisconsin, La Crosse WI 54601; (608) 785-6968. cmccoy@mail.uwlax.edu

ABBREVIATIONS:
AML = acute myeloid leukemia; APT = antigen presenting cell; CML = chronic myeloid leukemia; CRP = cryoprecipitate reduced plasma; CRYO = crytoprecipitated anti-hemophiliac factor; DC = dendritic cell; DCLHb = diaspirin cross-linked hemoglobin; DCLHb = diaspirin cross-linked hemoglobin; DLI = donor lymphocyte infusion; DMSO = dimethylsulfoxide; FFP = fresh frozen plasma; GVHD = graft-versus-host disease; GVL = graft-versus-leukemia; HbOC = hemoglobin-based oxygen carrier; HLA = human leukocyte antigens; IPMs = infusible platelet membranes; IVIG = intravenous immunoglobulin; LEHb = liposomes containing hemoglobin; PAP = prostatic acid phosphatase; PBMC = peripheral blood mononuclear cells; PEG = polyethylene glycol; POE = polyoxyethylene; PRBC = packed red blood cells; TPE = therapeutic plasma exchange.

INDEX TERMS: blood and platelet substitutes; blood component therapy; blood components; novel blood components.

Clin Lab Sci 2002;15(2):116

Michelle S Wright-Kanuth PhD CLS(NCA) is Associate Professor, Department of Clinical Laboratory Sciences at the University of Texas Medical Branch, Galveston TX.

Linda A Smith PhD CLS(NCA) is Professor and Graduate Program Director at the University of Texas Health Science Center at San Antonio, San Antonio TX.

Address for correspondence: Michelle S Wright-Kanuth PhD CLS(NCA), Department of Clinical Laboratory Sciences, 301 University Boulevard, Galveston, TX 77555-1140. (409) 772-3055, definitions, clauses *(409) 772-9470 (fax). mskanuth@utmb.edu*

Michele S Wright-Kanuth PhD is the Focus:Component Therapy guest editor.

Focus Continuing Education Credit: see pages 125 to 127 for learning objectives, test questions and application form.

LEARNING OBJECTIVES
At the end of the article the learner will be able to:

1. Identify the major diseases treated with each of the components discussed.
2. Discuss the preparation of DLI and why CD4 cells are retained, while CD8 cells are depleted.

3. Describe the general process of making a dendritic cell vaccine.
4. Describe the different types of red cell substitutes and differentiate between them.
5. Explain at least two of the possible ways in which intravenous immunoglobulins may interact with the immune system.
6. Explain why cryoprecipitate reduced plasma is effective in treating thrombotic thrombocytopenia.
7. List and describe the platelet products under development.

The role of the routine blood bank in providing therapeutic products has changed dramatically within the past five years. More emphasis is being placed on new and improved blood components and alternate uses for products. In addition, new sources for blood products have been researched, particularly in the field of blood substitutes. A review of the state of the art in some of these areas is timely. Donor lymphocyte infusion (DLI) is a new use for the lymphocyte fraction obtained from apheresis. Blood substitutes are coming of age and several sources of hemoglobin based oxygen carriers are currently in Phase III clinical trials. A promising immunotherapeutic approach to cancer therapy is dendritic cell vaccines. A number of dendritic cell vaccines are in Phase I and II clinical trials. FFP and cryosupernatent (the supernatent left after cryoprecipitate is made) are being used in therapeutic plasma exchange (TPE). Intravenous immunoglobulin (IVIG) has been used for over ten years; however, new applications for the use of this product are being studied. Cryopreservation and lyophilization of platelets and platelet substitutes are all being studied as remedies for the short shelf life of platelet components. These, then, are the areas that will be explored here.

DONOR LYMPHOCYTE INFUSION

Patients with chronic myeloid leukemia (CML) are offered the opportunity of a cure with allogeneic hematopoietic stem cell transplantation. Disease-free survival rates at five years reach 70% in transplanted patients. The cure is most likely partially due to the graft-versus-leukemia (GVL) effect mediated by donor-derived T lymphocytes. The GVL effect is primarily due to the derivation of the T cells from a healthy donor individual. The T cells in CML patients have been compromised by the tumor escape mechanisms that facilitate the growth of the CML in the patient.[1] When transplanted patients relapse after achieving remission, the administration of donor T lymphocytes is considered the initial response. DLI is achieved by collecting the transplant donor's white blood cells through apheresis. The donor cells are treated to deplete CD8+ T cells or other potentially harmful donor cells to prevent graft-versus-host disease (GVHD). The CD4+ T cells are necessary for the GVL reaction to occur.[2]

Various studies have shown that optimal numbers of CD4+ cells are required to induce remissions. Mackinnon and colleagues studied limiting the T cell dose given to induce remission. They found that most patients achieved remission at a dose of 1 x 10^7 T cells/kg body weight or above. However, the incidence of GVHD was greatly reduced at the lowest remission T cell dose of 1 x 10^7/kg.[3] Mandigers also studied a target T cell dose along with CD8+ cell depletion and saw similar results.[4]

Because the treatment of early CML relapse with DLI after stem cell transplant induces remission in the majority of patients studied, it is becoming an important treatment option. There is also promising data indicating that the use of DLI will affect remissions in relapsed acute myeloid leukemia (AML) patients.[5] These remissions may not be as common as those for CML patients, but some patients have stayed in remission for up to two years. In addition, DLI has been shown to have a graft-versus-myeloma effect in multiple myeloma patients.[6] As DLI becomes an increasingly documented and effective treatment, it will likely become the standard of care for such patients. Undoubtedly, blood centers and blood banks will be involved in the future procurement and cell manipulation of the DLI and in DLI storage and thawing, as they are becoming similarly involved in stem cell transplantation.

DENDRITIC CELL VACCINES

Dendritic cells (DCs) are the most potent of the antigen presenting cells (APCs). DCs can be found in most organs, the T cell areas of secondary lymphoid tissues, and circulating in the peripheral blood. During a normal immune response, the APCs phagocytize and process antigen and then present it along with MHC Class II molecules. The individual's T cells specific to that antigen will bind to the APC through the presented antigen and MHC molecule. The T cell will then be stimulated to respond and initiate the primary immune response. It is known that many tumor-associated antigens are weak immunogens and do not stimulate the immune response well. This lack of immunogenicity may be due to tolerance to self-antigens that is normally induced in the immune system during the education of T cells in the thymus.[7] Because it appears to be difficult to encourage natural antigen presentation of tumor-associated antigens, several groups of investigators have tried exposing the patient's DCs to exogenous sources of these antigens in vitro.

A phase I clinical trial was conducted in patients having prostate cancer. The investigators used mouse prostatic acid phosphatase (PAP) instead of the patient's own PAP to stimulate DCs obtained from the patient by peripheral blood leukaphoresis. The DCs and mouse PAP were incubated together overnight at 37 °C. The resulting antigen-treated DC vaccines were then reinfused into the patient. A T cell response to the self-PAP was then seen in 11 of 21 patients treated. Six of the patients showed stabilization of a previously progressing prostate cancer. No toxicity to the vaccine was seen.[7]

In another phase I clinical trial, solid tumors from children were reduced to single cell suspensions and cultured. The tumor cells were then lysed and the cell suspension, presumably containing tumor-associated antigens, was incubated with DCs collected from the patient by peripheral blood leukapheresis. The resulting DC

vaccines were now tumor specific for each patient's tumor. The vaccines were reinfused into the patient. No toxicity to the vaccine was seen in any of the 15 patients enrolled in the study. Six of the 15 patients were evaluated for immune response to the tumor lysate and three of the patients demonstrated a significant increase in response. The other nine patients did not have sufficient clinical material for evaluation.[8] Similar responses have been shown to occur in malignant gliomas.[9]

Metastaic melanoma tumor antigens having epitopes recognized by MHC Class I restricted cytotoxic T cells (CD8+) cells have also been identified. Thus, DC vaccines may not be the only useful cell vaccines. *In vitro* stimulation of peripheral blood mononuclear cells (PBMC) with tumor antigen derived epitopes and then returning the PBMC to the patient can induce a high number of tumor antigen reactive T cells in the patient.[10]

The advent of cell-based vaccines for cancer immunotherapy may be another area into which the future blood center and blood bank may expand. The preparation of these vaccines on a scale necessary for the treatment of multiple patients will require more space and effort than a research laboratory can provide.

RED CELL SUBSTITUTES

There are three types of red cell substitutes currently being studied for use in transfusion medicine. The goal is to enhance oxygen-carrying capacity for patients suffering from acute anemia due to blood loss. All of these have shown some promise for use in patients who refuse blood or in situations where blood is not readily available.

Hemoglobin-based oxygen carriers (HbOCs) are being studied as oxygen carrying substitutes for blood cells. They are purified cell-free hemoglobins, where the globin portion of the molecule has been modified chemically by conjugation, cross-linking or polymerizing. Modification increases the oxygen releasing ability of the hemoglobin. HbOCs fall into three categories: surface modified hemoglobins, cross-linked hemoglobins, and polymerized hemoglobins. *Surface modified Hbs* have molecules attached to the lysine residues on the surface of the hemoglobin molecule. Such attachments can be made using polyethylene glycol (PEG) and polyoxyethylene (POE). Such small molecules stabilize the hemoglobin and increase its molecular weight. Two of these products, PEG-Hb and pyridoxyl Hb-POE, are currently in clinical trials. *Cross-linked Hbs* consist of Hb subunits attached to each other using internal covalent bonds. Diaspirin cross-linked hemoglobin (DCLHb) is produced by using a reagent that cross-links the lysine residues in the Hba chains. These cross-links delay clearance of the free Hb from the circulation by stabilizing the Hb tetramer.[11] Most of the clinical studies have been done using human DCLHb. DCLHb trials were halted in 1999 due to increased mortality in some trial enrollees.[12] *Polymerized Hbs* are cross-linked at lysine residues with glutaraldehyde, which then has active aldehyde groups at both ends of the molecule. This allows polymers of Hb tetramers to form.[11] Human polymerized Hb is currently in clinical trials, as is a polymerized bovine Hb product. The bovine HbOC has completed phase III clinical trials and has been approved in South Africa for treatment of perioperative anemia in adult surgical patients.[12]

Perfluorocarbon-based red cell substitutes consist of carbon backbones highly substituted with fluorine. While they can dissolve large amounts of oxygen, the perfluorocarbons themselves are not water-soluble. To deliver them intravenously, they must be emulsified. This is accomplished using a surfactant such as a phospholipid. Perfluorocarbons were the original red cell substitutes and have potential due to the ability to synthesize them from non-biological sources. This not only allows large-scale production, but also eliminates the transmission of diseases to recipients. The perfluorocarbons are biologically inert; however, the phospholipids required to emulsify them are not, leading to complications when they interact with the immune system.

Three perfluorocarbons have entered clinical trials. The first to do so was marketed as Fluosol-DA and was licensed by the FDA in 1989.[13] However, it was withdrawn from the market due to lack of sales. Oxyfluor, an emulsion of perfluordichloroactane and egg yolk phospholipid with safflower oil, began clinical trials.[14] However, its development has been discontinued. The only perfluorcarbon that remains in clinical trials is Oxygent, an emulsion of perfluoroocytl bromide and egg yolk phospholipid. Oxygent is being studied for use in perioperative hemodilution to allow more extensive hemodilution.[15]

Hemoglobin-containing liposomes (LEHb) are formed using spheres of phosphatidylcholine to form a lipid bilayer that replicates the red cell membrane. Hb solution is then introduced inside the bilayer.[16] Since this is not a cell-free Hb, some of the potential for toxicity is diminished. Replicating the cellular format also results in a longer half-life in the circulation than the perfluorocarbons and most of the HbOC preparations. LEHb does, however, have a higher affinity for oxygen than some of the other red cell substitutes. Standardizing the size of liposomes is also problematic.[11]

PLATELET PRODUCTS AND SUBSTITUTES

Platelets participate in primary hemostasis by initially adhering to the vascular subendothelium and then using interactions between glycoproteins on the platelet surface and fibrinogen to initiate aggregation with the eventual formation of a platelet plug. Patients with low platelet counts may have petechiae or ecchymoses and those with extremely low platelet counts are at risk for spontaneous hemorrhage.

Major indications for transfusing platelets include prophylaxis in inheritable conditions that result in thrombocytopenia or dysfunctional platelets and to end active bleeding in thrombocytopenic patients. A little over 50 years ago, the only sources of platelets were from fresh whole blood or platelet rich plasma. Since that

time, the first separation of platelets by centrifugation yielded a product with a shelf life of about two hours and refrigeration of platelet concentrates at 1 °C to 6 °C provided products with a shelf-life of 24 hours. Thirty years ago we began to store platelets only at room temperature. The advent of new plastic containers allows platelets to be kept for up to five days before outdating.

In recent years platelet transfusion therapy has focused on the use of two products—random donor pooled platelets or apheresis platelets. Obtaining a 'pooled platelet' preparation is a two-step procedure. First, one unit of platelets is harvested from a unit of red cells. Then four to six of these individual units (from different donors) are 'pooled' together in a single pack to be given to a thrombocytopenic patient. Apheresis platelets, on the other hand, are those collected from a single donor using cell separator instrumentation. As blood cycles through the machine, platelets are removed and all other blood constituents returned to the donor. The amount of platelets collected with this procedure represents the equivalent of four to six units of random donor platelets. Leukoreduction filters can be used with either of these to remove the majority of white blood cells before infusion and therefore decrease the risk of sensitization to human leukocyte antigens (HLA), symptoms caused by production of cytokines in the stored PRBCs, or the risk of transmission of cytomegalovirus.

The risks associated with sensitization include development of alloantibodies to HLA antigens or to platelet-specific antigens. These antibodies may cause the patient to experience a febrile non-hemolytic transfusion reaction or become refractory to a platelet transfusion. A patient who is 'refractory' does not have the expected increment in the post-transfusion platelet count due to antibody-mediated destruction of transfused platelets. In some cases in which the patient has developed antibodies to HLA antigens and become refractory, apheresis platelets that are HLA matched to the patient's antigens may be used. A summary of problems and biological risks associated with platelet transfusions are listed in Table 1.

Platelets have short shelf life and also develop changes in functional ability during storage. Even with the short shelf life, storage at room temperature increases the risks of bacterial growth. Over the past 40 years, unsuccessful attempts were made to cryopreserve or lyophilize platelets to overcome these problems and today the search for alternative ways of preserving platelets or creating platelet substitutes continues. Numerous criteria that novel platelet products or platelet substitutes should meet have been described in the literature and some of these are listed in Table 2.[17,18] However, just as with the functional comparison between packed red blood cells and blood substitutes, these products are designed for short term treatment of active bleeding and do not possess all the functions of fresh human platelets.

Products under development to augment platelet function, decrease risks associated with transfusion, or to substitute for platelets include: cryopreservation with or without synthetic additives, lyophilized platelets, photochemically treated platelets, infusible platelet membranes, and fibrinogen coated albumin microspheres.[17,18,19]

Cryopreservation

Platelets suspended in dimethylsulfoxide (DMSO) at -80 °C have been preserved up to ten years and represent the 'gold standard' of preserved platelet products. During the thawing and post-thaw processing however, these platelets develop functional and morphologic defects. Although there is some loss of functional activity when compared to fresh platelets, cryopreserved platelets do demonstrate a reduced level of primary hemostatic activity.[17,20-22] The numbers of platelets that are recovered is about 75% of the original number and they have a short circulation time in vivo.[17,20] Because of the complexities of storing, processing, and thawing frozen platelets, the current use is limited. Several studies have been done using a decreased concentration of DMSO with a platelet-stabilizing solution (ThromboSol™) to decrease problems such

Table 1. Biological risks associated with platelet transfusion

Alloimmunization (development of antibodies to HLA or platelet specific antigens)
- –refractory state
- –febrile non-hemolytic transfusion reactions

Bacterial, viral or parasitic contamination
- –disease transmission
- –septic shock

Immune system effects [uncommon]
- –immunosuppression
- –graft-versus-host disease

Table 2. Selected criteria for platelet substitutes or novel platelet products

Function hemostatically as 'live platelets'
- –attach to vascular surfaces
- –provide a procoagulant surface

Avoid initiating consumptive coagulopathy or thrombosis

Not transmit infectious diseases

Be non-immunogenic

Have a long shelf life and simple storage requirements

Be easy to prepare

as decreased recovery and short circulation time that occur with current methods of cryopreserving platelets. This solution inhibits platelet activation pathways and protects against cold storage lesions. Results of these studies have shown higher recovery rates and longer survival times than with the 6% DMSO method as well as fewer processing steps.[23,24] There are investigations under way to develop methods that do not require processing after freezing and can be directly infused after thawing.

Lyophilized platelets

Lyophilized platelets are created after treatment with a paraformaldehyde solution and then freeze-dried.[25,26] Specific advantages of this product include storage measured in years instead of days, reduced storage space and true sterility. Once rehydrated, they appear to retain structural integrity and attach only to damaged subendothelial surfaces.[25] They will also change shape and extend pseudopods in preparation for plug formation.[27] Thus it appears that the GpIb receptor which binds to vonWillebrand factor and then to collagen of the injured surface is not significantly affected by freeze-drying.[25,26] Data for other receptor complexes such as GPIIbIIIa , which is the fibrinogen receptor, are not as clear cut. The number of these receptors and their function appears to be diminished but not entirely eliminated.[27] There appears to be minimal risk for developing systemic thrombosis after administration. Animal studies have shown that the rehydrated lyophilized platelets have hemostatic efficiency–measured by bleeding time–similar to that of fresh platelets; however this effect continues for only several hours.[25]

Infusible platelet membranes

Infusible platelet membranes (IPMs) are manufactured from outdated platelet units in an attempt to provide a stable product that mimics the actions of the platelet-derived microparticles.[28] Platelet derived microparticles (microvesicles) are the particles that form spontaneously from a platelet during collection and processing of components. They have been found in platelet concentrates, fresh frozen plasma, and cryoprecipitate.[19] They appear to have the ability to function as a platelet—they are procoagulant active, adhere to vascular subendothelium, and enhance platelet adhesion to form a primary hemostatic plug. Studies have shown that the IPMs do retain some function of GpIb receptor and bind to vWF and can initiate local fibrin formation, but much of the GpIIb/IIIa is lost.[29,30] One application for IPMs may be in patients who are refractory to platelet transfusions and for whom finding HLA matched plateletpheresis donors is difficult. One problem appears to be a relatively short life (less than 24 hours) in vivo.[28]

Miscellaneous microspheres

There are a number of products that use formaldehyde fixed platelets, liposomes, or 10% albumin spheres as a basis on which to coat fibrinogen or platelet membrane glycoproteins. Results from some of the pre-clinical trials show that these products appear to be able to enhance the adhesion of platelets and formation of aggregates but in vivo stability remains a problem.

Contamination of platelets

A peripheral but important issue with transfusion of human pooled platelets is the risk of transfusion transmitted diseases—especially bacterial–and the associated potential for septic shock and death. While serologic tests for transfusion transmitted viruses such as hepatitis B have reduced the incidence of viral transmission significantly, there have not been concurrent advances for detecting bacterial contamination. Platelets stored at room temperature create an ideal incubation environment for growth of bacteria. Studies indicate that 1 in 2,000 to 1 in 3,000 platelet units are bacterially contaminated, with sepsis occurring with about 1/6 of the contaminated units transfused.[31,32,33] Units at the end of the four to five day storage period are the most likely to be contaminated.[31,34] Contamination can originate from occult bacteremia in the donor, induction of skin bacteria such as *Staphylococcus epidermidis* during phlebotomy, or contaminated collecting devices. Detection of contaminated units is difficult and a number of methods have been proposed to determine contamination prior to transfusion.[31,35] These include examination of Gram's stains of units at day four or five, or surrogate methods such as measuring low pH or glucose levels of units with a urine dipstick. The major disadvantage of these methods is that they are not sensitive and require large numbers of organisms for detection. One study showed that detection methods must be sensitive enough to detect 100 CFU/ml by day three of storage.[36] Several researchers have evaluated an automated culture system which could detect organisms with concentrations as low as 10 to 100 colony forming units /mL in 9 to 26 hours.[36,37] The results indicated that short-time culture in automated systems may be useful in screening platelet units for contamination. Methods used to prevent contamination have also been investigated. One method is use of a photochemical agent and ultraviolet light (UV) to inactivate bacteria and viruses in conventional platelet units. The chemical, in the presence of UV light, will bind to DNA to prevent transcription and replication.[38,39,40] Studies have shown this treatment will inactivate high concentrations of bacteria and viruses without significantly affecting the hemostatic activity of the platelets.[39,40]

Intravenous immunoglobulin

Once the fractionation of immunoglobulins was successfully performed in the 1940s, the use of immunoglobulins (especially gamma globulin) became an established method of providing protective, passive immunity for some diseases. However, intravenous administration was not possible because the method of preparation often resulted in a variety of patient side effects including anaphylaxis. During the 1980s and 1990s changes in manufacturing allowed fractionation of the product into IgG portions that could be solublized and used intravenously.[41]

Intravenous immunoglobulin (IVIG) is made from large pools of donor plasma (hundreds to thousands of donors). This polyclonal preparation contains 90% to 98% IgG and small amounts of IgA and IgM.[41] Bacteria are removed by filtration and viral agents are inactivated by a variety of mechanisms. This pooling of donor

plasma provides a diversity of antibodies that have led to the use of IVIG for treatment in a wide spectrum of diseases. In contrast, monoclonal antibodies have a use that is limited to a specific disease. For example anti-tumor necrosis factor can be used as adjunct treatment for rheumatoid arthritis.

The mechanism of how IVIG works is not completely known. Studies with specific diseases and animal models have shown that the therapeutic action includes one or more of the mechanisms listed in Table 3.[42-45] The most commonly recognized mechanisms appear to be those of competition for binding sites on the Fc receptors of phagocytic cells and the binding of anti-idiotypic antibodies (antibodies to human antibodies) to autoantibodies by attaching to the Fab portion of the immunoglobulin molecule.

IVIG was first licensed in the early 1980s to be used as treatment for primary immunodeficiency diseases characterized by hypogammaglobulinemia and/or recurrent infection. For individuals with these diseases, it provides a source of antibodies and decreases the incidence and severity of infections in this population. After its initial use as a replacement therapy, it was also found to have an immunomodulatory effect and its use expanded to include selective treatment for hematologic, inflammatory, and infectious diseases that have an immunologic component. The FDA has approved the use of IVIG for treatment of more than 30 disease conditions including primary immune deficiency, B-cell chronic lymphocytic leukemia, idiopathic thrombocytopenia purpura (ITP), pediatric human immunodeficiency virus infection, Kawasaki syndrome, and neuroimmunologic diseases such as Guillain-Barre syndrome and selected obstetric conditions. In addition, it has been approved for use in allogeneic bone marrow transplant patients to prevent GVHD as well as infections. The relative success of IVIG in many conditions, however, has also led to use in treating many other conditions for which it has not been approved.

One of the first conditions in which IVIG was recognized as effective treatment was ITP. In ITP the two major mechanisms for action of IVIG include blocking Fc receptors on splenic macrophages and reaction of anti-idiotypic antibodies with autoantibodies. It appears that more immediate effects are due to inhibition of the RES and the anti-idiotypic antibodies may function in a long term protective role.[43,46] A study of antibody coated platelets showed that the Fc fragments of immunoglobulins in IVIG gave protection by inducing expression of an inhibitory receptor on effector cells. This decreased or prevented the clearance of the antibody-coated platelet.[46] In another study, high-dose IVIG therapy accelerated clearance of autoantibodies but could only explain 20% to 40% of the decrease in autoantibody concentration after therapy.[44] When ITP occurred in pregnant women the IVIG was also effective in decreasing platelet damage in the fetus.

Table 3. Potential mechanisms of IVIG

Binding to complement proteins
Inhibition and regulation of cytokine action
Interference with antigen recognition by T cells
Activation of neutrophils
Competition for binding to Fc receptors
Interaction with superantigens
Binding to autoantibodies

Another obstetrically-related condition in which IVIG has been used is neonatal alloimmune thrombocytopenia (NAIT). In this disease, the mother develops antibodies against fetal platelet antigens, most commonly the Human Platelet Antigen 1a, formerly known as PL^{a1}. Infants with this condition are born with clinical indications of moderate or severe thrombocytopenia and may be at risk for intracranial hemorrhage.[47] As with other fetal-maternal alloimmune conditions, the risk to the fetus and the severity of the condition can become more severe with each subsequent pregnancy. Once the condition has been identified, IVIG can be given to the mother during the pregnancy. IVIG crosses the placenta and provides protection to the fetus. It may also inhibit maternal immunoglobulin synthesis through a feedback mechanism or inhibit transport of the maternal antibodies across the placenta. This is effective in decreasing platelet destruction in 50% to 80% of cases.[47,48] A study by Gaddipati linked the initial fetal platelet count to the subsequent efficacy of IVIG therapy. If the fetal platelet count was >20,000/microliter then approximately 89% of future counts were above that level. If the platelet count was <20,000 then only 51% had an increased count after the IVIG.[49]

Although the use of Rh immunoglobulin (RhIg) has successfully reduced the number of cases of Rh_o (D) hemolytic disease of the newborn due to anti-D, there are some RhIg failures. In addition hemolytic disease of the newborn may be due to antibodies to other blood group system antigens. In cases where the maternal antibody is extremely high and intrauterine transfusion is unable to be performed, IVIG has been used to decrease maternal antibody titer.[47,50]

Because neonates have an immature immune system and may be at increased risk for infection, the use of IVIG in treating sepsis has also been studied. Studies as well as a meta-analysis of studies of IVIG use in treating neonatal sepsis showed that IVIG may be of significant benefit in addition to standard treatment for neonates early in the onset of sepsis but had minimal benefit when used prophylactically.[51,52]

Autoimmune diseases are another area in which IVIG has been used. These diseases present challenges for treatment. One is bal-

ancing the suppression of the immune system against the risks of infection. Another challenge is dealing with exacerbation or crises in the disease when the antibody titer reaches very high levels. IVIG has been useful in treating autoimmune diseases such as myasthenia gravis. The culprit in this condition is an autoantibody to acetylcholine receptors that interferes with nerve impulse transmission. It is characterized by weakness of voluntary muscles and affects both women and men of any age. Although drugs are the usual long-term therapy, IVIG is a temporary treatment to decrease antibody production. One study determined that there may be some efficacy in using IVIG as a replacement treatment for patients with Guillain-Barre syndrome who could not undergo plasma exchange.[53]

Kawasaki syndrome is a leading cause of acquired heart disease in North America and Japan. Its cause is unknown although an infectious agent has been suggested. It is a self-limiting disease that leads to coronary artery lesions.[54] Therapy with IVIG along with aspirin during the first ten days of the illness decreases risk of coronary artery damage. There is some evidence to suggest that the IVIG may also decrease circulating cytokines that mediate much of the damage.

There are multiple studies of other possible applications of IVIG therapy in diseases with infectious or immunologic origins.[54-56] In pediatric HIV patients who have hypergammaglobulinemia but the impaired ability to produce specific antibodies, IVIG was used to decrease episodes of acute pneumonia but unfortunately did not increase survival.[54] Another study looked at whether IVIG could be used in treatment of acute rheumatic fever.[55] Despite the underlying immunologic basis for acute rheumatic fever, IVIG showed no effect on clinical progress or other disease parameters. In another study IVIG was studied as a supplemental treatment in patients with sepsis and septic shock.[56] Again there was no overwhelming improvement in those who received the IVIG, but it appeared to decrease morbidity and mortality in some patients when used as part of the treatment protocol.

COMPONENTS USED IN THERAPEUTIC PLASMA EXCHANGE

Fresh frozen plasma

Fresh frozen plasma (FFP) is the component created when plasma is removed from a unit of blood and frozen at –18 °C within eight hours after collection. It contains stabile and labile coagulation factors, immunoglobulins, and proteins and has been used in treating a number of conditions (Table 4). One of the most common ways it is used is in therapeutic plasma exchange (TPE). TPE involves removal of a patient's plasma and a return of the cellular elements in a liquid medium replacement. FFP is the preferred medium over crystalloids such as physiologic saline or albumin because it is not only a volume expander, but is also a source of proteins and immunoglobulins. Over the years, TPE has become accepted therapy for a number of diseases such as cryoglobulinemia, myasthenia gravis, Guillain-Barre syndrome, and thrombotic thrombocytopenic purpura (TTP).[57-60] It has been used with varying degrees of success in diseases such as cold agglutinin disease, systemic vasculitis, chronic inflammatory demyelinating polyneuropathy, hemolytic uremic syndrome (HUS), and to remove high levels of antibody in pregnant women when uterine transfusion cannot be accomplished. Although TPE generally will not cure the underlying condition, the procedure will often temporarily alleviate symptoms by decreasing the concentration of the underlying problematic plasma component. Table 5 lists some of the specific components that can be removed by TPE.

The disease in which TPE has been used most successfully is TTP and this disease will be used as an example of how TPE may alleviate underlying conditions.

TTP is characterized by a pentad of symptoms including thrombocytopenia, microangiopathic hemolytic anemia, fever, neurological symptoms, and renal dysfunction. It may manifest as a single acute episode or a chronic relapsing condition. The cause of the disease is unknown but it may be triggered by a variety of conditions including pregnancy and infections. In TTP a combination of endothelial cell damage and platelet aggregation agents results in microthrombi and a consumptive thrombocytopenia. Research has shown that in contrast to the usual platelet plugs that are composed of platelets and fibrinogen, those in TTP are composed of platelets and ultra large multimers of vonWillebrand Factor (uLvWF).[61,62] The presence of these uLvWF multimers led researchers to investigate why these multimers were present. Findings indicate it may be an absence of a vWF cleaving protein in the plasma of patients with TTP. Plasma exchange using FFP removes some of the multimers and provides a source of enzyme, however, the FFP itself remains a source of vWF.[61,62] Although TPE treatments are successful in many cases, there were a number of patients who did not respond. The research of causes and the identification of vWF as a possible cause led to the use of another blood component in TPE—cryoprecipitate reduced plasma (cryosupernatant or cryo-poor plasma).[63-66]

Table 4. Indications for use of fresh frozen plasma

Consumptive coagulopathies such as disseminated intravascular coagulation (DIC)
Multiple coagulation factor deficiencies
Liver disease
Dilutional coagulopathies such as those seen in massive transfusion
Thrombotic thrombocytopenia purpura (TTP)
Deficiencies of Protein C, Protein S

Cryoprecipitate reduced plasma

Cryoprecipitate reduced plasma (CRP) is the plasma remaining once cryoprecipitate has been prepared from the FFP. Preparation of the cryoprecipitate removes much of the fibrinogen, Factor VIII, and vWF from the plasma. However, the vWF enzyme remains. This eliminates a source of vWF for multimer formation and provides the patient with the deficient enzyme to degrade the existing multimers. TPE is the only disease in which the component is used as therapeutic treatment.

The major type of transfusion reaction associated with the use of either component in TPE is allergic (reaction to plasma proteins). In a few cases these may be anaphylactic when the recipient lacks serum IgA and has developed antibodies to plasma IgA (anti-IgA antibodies). The risks are relatively small in comparison to the high mortality of untreated TTP.

In summary, this article has addressed the use of some novel component preparations in treatment of specific disease conditions and the status of substitutes for conventional components. The constantly changing technology, the increased knowledge about how the immune system functions, the increased numbers of patients needing component support, and the spectrum of transfusion-transmitted diseases will assure that the research and clinical trials of new components will increase in the coming years.

REFERENCES

1. Ochsenbein AF, Klenerman P, Karrer U, and others. Immune surveillance against a solid tumor fails because of immunological ignorance. Proc Natl Acad Sci USA 1999;96:2233.
2. Dazzi F, Szydlo RM, Goldman JM. Donor lymphocyte infusions for relapse of chronic myeloid leukemia after allogeneic stem cell transplant: where we now stand. Exp Hematol 1999;27:1477-86.
3. Mackinnon S, Papadopolous EP, Carabasi MH, and others. Adoptive immunotherapy evaluating escalating doses of donor leukocytes for relapse of chronic myeloid leukemia following bone marrow transplantation: separation of graft-versus-leukemia responses from graft-versus-host disease. Blood 1995;86:1261-8.
4. Mandigers CM, Meijerlink JP, Raemaekers JM, and others. Graft-versus-lymphoma effect of donor leucocyte infusion shown by real-time quantitative PCR analysis of t(14:18). Lancet 1998;352:1522-3.
5. Kolb HJ. Donor leukocyte infusions for treatment of leukemic relapse after bone marrow transplantation. EBMT Immunology and Chronic Leukemia Working Parties. Vox Sang 1998;74:321-9.
6. Mackinnon S. Who may benefit from donor leucocyte infusions after allogeneic stem cell transplantation? Brit J Haematol 2000;110:12-7.
7. Fong L, Brockstedt D, Benike C, and others. Dendritic cell-based xenoantigen vaccination for prostate cancer immunotherapy. J Immunol 2001;167:7150-6.
8. Geiger JD, Hutchinson RJ, Hohenkirk LF, and others. Vaccination of pediatric solid tumor patients with tumor lysate-pulsed dendritic cells can expand specific T cells and mediate tumor regression. Cancer Res 2001;61:8513-9.
9. Soling A, Rainov NG. Dendritic cell therapy of primary brain tumors. Mol Med 2001;7:659-67.
10. Wang E, Phan GQ, Marincola FM. T-cell directed cancer vaccines: the melanoma model. Expert Opin Biol Ther 2001;1:277-90.
11. Stowell CP, Levin J, Spiess BD, and others. Progress in the development of RBC substitutes. Transfusion 2001;41:287-99.
12. Standl T. Haemoglobin-based erythrocyte transfusion substitutes. Expert Opin Biol Ther 2001;1:831-43.
13. Kerins DM. Role of perfluorocarbon Fluosol-DA in coronary angioplasty. Am J Med Sci 1994;307:218-21.
14. Cochran RP, Kunzelman KS, Vocelka CR, and others. Perfluorocarbon emulsion in cardiopulmonary bypass prime reduces neurologic injury. Ann Thorac Sur 1997;63:1326-32.
15. Wahr JA, Trouwborst A, Spense RK. A pilot study of the effects of perflubron emulsion, AF0104, on mixed venous oxygen tension in anesthetized surgical patients. Anesth Analg 1996;82:103-7.
16. Rabinovici R, Rudolph AS, Vernick J, and others. Lyophilized liposome encapsulated hemoglobin: evaluation of hemodynamic, biochemical and hematologic responses. Crit Care Med 1994;22:480-5.
17. Lee DH, Blajchman MA. Novel treatment modalities: new platelet preparations and substitutes. Br J Haematol 2001;114:496-505.
18. Silberman S. Platelets: preparations, transfusion, modifications and substitutes. Arch Pathol Lab Med 1999;123:889-94.
19. Blajchman MA. Platelet substitutes. Vox Sang 2000;78(S2):183-6.
20. Melaragno AJ, Carciero R, Feingold H, and others. Cryopreservation of human platelets using 6% dimethyl sulfoxide and storage at –80 degrees C. Effects of two years of frozen storage at –80 degrees C and transportation in dry ice. Vox Sang 1985;49:245-58.
21. Rothwell S, Maglasang P, Reid TJ, and others. Correlation of in vivo and in vitro functions of fresh and stored human platelets. Transfusion 2000;40:988-93.
22. Barnard MR, MacGregor H, Ragno G, and others. Fresh, liquid-preserved, and cryopreserved platelets: adhesive surface receptors and membrane procoagulant activity. Transfusion 1999;8:880-8.
23. Currie LM, Lichtiger B, Livesey SA, and others. Enhanced circulatory parameters of human platelets cryopreserved with second-messenger effectors: an in vivo study of 16 volunteer platelet donors. Br J Haematol 1999;105:826-31.
24. Pedrazzoli P, Noris P, Perotti C, and others. Transfusion of platelet concentrates cryopreserved with ThromboSol plus low-dose dimethylsulphoxide in patients with severe thrombocytopenia: a pilot study. Br J Haematol 2000;108:653-9.
25. Bode AP, Read MS. Lyophilized platelets: continued development. Transfus Sci 2000;22:99-105.
26. Bode AP. Preclinical testing of lyophilized platelets as a product for transfusion medicine. Transfus Sci 1995;16:183-5.

Table 5. Removal of specific substances with therapeutic plasma exchange

Factor	Disease or condition
Platelet aggregating factors	TTP
Immune complexes	SLE
Antibodies causing hyperviscosity	Waldenstrom's macroglobulinemia
Autoantibodies	SLE
Alloantibodies	Hemolytic disease of the newborn
Antibodies blocking receptors	Myasthenia gravis

27. Fischer TH, Merricks EP, Russell KE, and others. Intracellular function in rehydrated lyophilized platelets. Br J Haematol 2000;111:167-74.
28. Chao FC, Kim BK, Houranieh AM, and others. Infusible platelet membrane microvesicles: a potential transfusion substitute for platelets. Transfusion 1996;36:536-42.
29. Galan AM, Bozzo J, Hernandez MR, and others. Infusible platelet membranes improve hemostasis in thrombocytopenic blood: Experimental studies under flow conditions. Transfusion 2001;40:1074-80.
30. Graham SS, Gonchoroff NJ, Miller JL. Infusible platelet membranes retain partial functionality of the platelet GPIb/IX/V receptor complex. Am J Clin Path 2001;115:144-7.
31. Yomtovian R, Lazarus HM, Goodnough LT, and others. A prospective microbiologic surveillance program to detect and prevent the transfusion of bacterially contaminated platelets. Transfusion 1993;33:902-9.
32. Engelfriet Co, Reesink HW, Blajchman MA, and others. Bacterial contamination of blood components. Vox Sang 2000;78:59-67.
33. Sazama K. Bacteria in blood for transfusion. A review. Arch Pathol Lab Med 1994;118:350-65.
34. Morrow JF, Braine HG, Kickler TS, and others. Septic reactions to platelet transfusions. A persistent problem. JAMA 1991;266:555-8.
35. Barrett BB, Andersen JW, Anderson KC. Strategies for the avoidance of bacterial contamination of blood components. Transfusion 1993;33:228-33.
36. Brecher ME, Means N, Jere CS, and others. Evaluation of an automated culture system for detecting bacterial contamination of platelets: An analysis with 15 contaminating organisms. Transfusion 2001;41:477-82.
37. Liu HW, Yeun KY, Cheng T, and others. Reduction of platelet transfusion-associated sepsis by short-term bacterial culture. Vox Sang 1999;77:1-5.
38. Corash L. Inactivation of viruses, bacteria, protozoa, and leukocytes in platelet concentrates. Current research perspectives. Transf Med Rev 1999;13:18-30.
39. Lin L, Cook DN, Wiesehahn GP, and others. Photochemical inactivation of viruses and bacteria in platelet concentrates by use of a novel psoralen and long-wavelength ultraviolet light. Transfusion 1997;37:423-35.
40. VanRhenen D, Gullikkson H, Pamphilon D, and others. S-59(HelinxTM) photochemically treated platelets are safe and effective for support of thrombocytopenia: Results of the Eurosprite phase 3 trial. Blood 2000;96:819.
41. Swenson MR. Autoimmunity and immunotherapy. J IV Nurs 2000;23 supplement:S8-S13.
42. Teeling, JL, Bleeker WK, Rigter GMM, and others. Intravenous immunoglobulin preparations induce mild activation of neutrophils in vivo via triggering of macrophages – studies in a rat model. Br J Haematol 2001;112:1031-40.
43. Crow AR, Song SI, Semple JW, and others. IVIG inhibits reticuloendothelial system function and ameliorates murine passive-immune thrombocytopenia independent of anti-idiotype reactivity. Br J Haematol 2001;115:679-86.
44. Bleeker WK, Teeling JL, Hack CE. Accelerated autoantibody clearance by intravenous immunoglobulin therapy: studies in experimental models to determine the magnitude and time course of the effect. Blood 2001;98:3136-42.
45. Sacher R. Intravenous immunoglobulin consensus statement. J Allergy Clin Immunol 2001;108 supplement:S139-46.
46. Samuelsson A, Towers TL, Ravetch JV. Anti-inflammatory activity of IVIG mediated through the inhibitory Fc receptor. Science 2001;291:484-6.
47. Branch DW, Porter TF, Paidas MJ, and others. Obstetric uses of intravenous immunoglobulin: successes, failures, and promises. J Allergy Clin Immunol 2001;108 supplement:S133-8.
48. Bussel JB, Barques RL, Lynch L, and others. Antenatal management of alloimmune thrombocytopenia with intravenous immune globulin: a randomized trial of the addition of low dose steroid to intravenous immune globulin. Am J Obstet Gynecol 1996;174:1414-23.
49. Gaddipati S, Berkowitz RL, Lembet AA, and others. Initial fetal platelet counts predict the response to intravenous gammaglobulin therapy in fetuses that are affected by PLA1 incompatibility. Am J Obstet Gynecol 2001;185:976-80.
50. Porter TF, Silver RM, Jackson G, and others. Intravenous immune globulin in the management of severe RH D hemolytic disease. Obstetrical and Gynecolgical Survey 1997;52:193-7.
51. Hill HR. Additional confirmation of the lack of effect of intravenous immunoglobulin in the prevention of neonatal infection. J Pediciatr 2000;137:595-97.
52. Jenson HB, Pollock BH. Meta-analysis of the effectiveness of intravenous immune globulin for prevention and treatment of neonatal sepsis. Pediatrics 1997;99:E2.
53. Raphael JC, Chevret S, Harboun M, and others. Intravenous immune globulins inpatients with Guillain-Barre syndrome and contraindications to plasma exchange: 3 days versus 6 days. J Neurol, Neurosurgery, and Psychiatry 2001;71:235-8.
54. Nowak-Wegrzyn A, Lederman HM. Supply, use, and abuse of intravenous immunoglobulin. Curr Opin Pediatr 1999;11:533-9.
55. Voss LM, Wilson NJ, Neutze JM, and others. Intravenous immunoglobulin in acute rheumatic fever: A randomized controlled trial. Circ 2001;103:401-6.
56. Werdan K. Pathophysiology of septic shock and multiple organ dysfunction syndrome and various therapeutic approaches with special emphasis on immunoglobulins. Ther Apher 2001;5:115-22.
57. Moake JL. Thrombotic thrombocytopenic purpura. Thromb Haemost 1995;74:240-5.
58. Bosch T, Buhmann R, Lennertz A, and others. Therapeutic plasma exchange in patients suffering from thrombotic microangiopathy after allogeneic bone marrow transplantation. Ther Apher 1999;3:252-6.
59. Parry GJ. When is antibody testing useful and what therapy is effective in immunomediated neuropathies? Adv Neuroimmunol 1995;2:18-22.
60. Plasma exchange/Sandoglobin Guillain-Barre syndrome trial group. Randomized trial of plasma exchange, intravenous immunoglobulin, and combined treatments in Guillain-Barre syndrome. Lancet 1997;349:225-30.
61. Furlan M, Robles R, Galbusera M, and others. Von Willebrand factor-cleaving protease in thrombotic thrombocytopenic purpura and the hemolytic-uremic syndromes. N Engl J Med 1998;339:1578-84.
62. Furlan M, Robles R, Solenthaler M, and others. Deficient activity of Von Willebrand factor-cleaving protease in chronic relapsing thrombotic thrombocytopenic purpura. Blood 1997;89:3097-103.
63. Rock G, Shumak KH, Sutton DM, and others. Cryosupernatant as replacement fluid for plasma exchange in thrombotic thrombocytopenic purpura. Members of the Canadian Apheresis Group. Br J Haematol 1996;94:383-6.
64. Rock G, Anerson DR, Benny WB, and others. Changes in vWF levels in TTP patients treated with cryosupernatant versus fresh frozen plasma. Br J Haematol 1998;102(I-1):369.
65. Blackall, DP, Uhl L, Spitalnik SL, and others. Cryoprecipitate-reduced plasma: rational for use and efficacy in the treatment of thrombotic thrombocytopenic purpura. Transfusion 2001;41:840-4.
66. Ellis J, Theodossiou C, Schwarzenberger P. Treatment of thrombotic thrombocytopenic purpura with the cryosupernatant fraction of plasma: a case report and review of the literature. Am J Med Sci 1999;318:190-3.