
91 Advances in Transfusion Safety

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SUMMARY

Although transfusion is now extremely safe, the tools of molecular biology are continually being harnessed to improve diagnosis and therapy. Viral genome testing has been introduced in the developed world for HIV and HCV, to detect donors in the infectious “window period” before sero-conversion. Pathogen inactivated fresh frozen plasma and platelets are already available, but alloimmunization has halted trials of pathogen-inactivated red cells. Development of synthetic oxygen carriers has included perfluorocarbons, and crosslinked, polymerized or mutated human or bovine hemoglobin, either free or encapsulated. No perfect replacement for the human red cell is yet on the horizon.

Key Words: Bacterial transmission; blood substitutes; perfluorocarbons; hemoglobin solutions; nucleic acid viral testing; pathogen inactivation; transfusion safety; transfusion-transmitted parasites; transfusion-transmitted viruses.

INTRODUCTION

For many years, there were few changes to the way blood for transfusion was processed and tested. Whole blood donations were separated into red cells, platelets, and plasma, while virus testing usually consisted of serological assays for either viral antigens or antibodies in the donor. The viral safety of blood, already high in the more affluent parts of the world, potentially took a quantum leap with the development of rapid, automatable genome amplification assays on the one hand, and pathogen inactivation techniques on the other. Meantime, there was a search to find a way of avoiding human blood transfusion altogether, by developing alternative ways of transporting oxygen from the lungs to the tissues. This chapter outlines the rapid change in these fields in the last two decades, and attempts to set these into the context of policy making for national blood supplies.

RED CELL SUBSTITUTES

WHAT IS NEEDED AND WHY “Artificial blood,” meaning a synthetic oxygen carrier, has been the holy grail of transfusion research for decades. Innovation in this area was originally driven by military requirements for a long shelf life battlefield solution that would need neither refrigeration nor matching with the recipient. The publicity surrounding transfusion-transmitted infections since the 1980s (HIV, hepatitis B and C, West Nile virus [WNV], and

now prions) provided a new impetus to these developments. Now many countries face chronic blood shortages. In developed countries, this is primarily because of an imbalance between the blood requirements of an aging population and a diminishing donor base, whereas in the developing world, lack of infrastructure to collect, test, and store blood safely is a major issue. So the need for alternatives to human blood remains.

However, the task facing developers of oxygen carriers is daunting. The human red cell is a miracle of evolution; its shape, the gas exchange properties of its membrane, and the complex interactions between oxygen, CO₂, 2,3 diphosphoglycerate (2,3 DPG), the vessel wall, and hemoglobin itself make the creation of a substitute a challenging task. Moreover, it has become increasingly clear that a “one size fits all” solution will not work, and that the specification of a product for transfusion dependent patients could differ from what is needed for acute hemorrhage. Virtually all development in this area has focused on acute replacement, and the term “bridge oxygenator” has appeared to describe solutions designed to maintain tissue oxygenation until the patient can receive conventional red cell transfusion.

Two classes of compounds have been extensively studied as potential oxygen carriers: perfluorocarbons and hemoglobin solutions. Each type has its merits and problems (Table 91-1). A number of promising first generation compounds of both types failed clinical trials, forcing a return to basic research on the events in the microvasculature that determine outcome in acute severe blood loss. Much more is now understood concerning the optimal characteristics of synthetic oxygen carriers, and although none is yet licensed in either Europe or North America, second generation products may perhaps fulfill early hopes.

PHYSIOLOGICAL RESPONSE TO HEMORRHAGE In acute hemorrhage, physiological responses designed to preserve the blood supply to vital organs at the expense of peripheral perfusion are automatic. Peripheral vasoconstriction is mediated by autonomic reflexes. At less than 50% blood loss, fluid shifts into circulation to maintain blood volume, so hematocrit and viscosity fall. This reduces the shear forces exerted on the endothelium, resulting in a reduction of production and release of natural vasodilators such as nitric oxide (NO) and prostacyclin. Peripheral resistance therefore rises further, and tissue blood flow slows even more, until a point is reached when oxygen supply is insufficient for tissue demands. Then anaerobic respiration takes over, leading to buildup of CO₂ and lactic acid. Once established, this state of metabolic acidosis is difficult to treat.

Table 91-1
Advantages and Disadvantages of Hemoglobin Solutions and Perfluorocarbons

| <i>Advantages</i> | <i>Disadvantages</i> |
|--|---|
| <i>Hemoglobin solutions</i> | |
| High capacity for O ₂ and CO ₂ | Short plasma half-life |
| Functions at physiological pH | Potential renal toxicity |
| Oncotic activity | Vasoactivity |
| Absent red blood cell antigens | Auto-oxidation |
| Prolonged shelf life | |
| Virus inactivation possible | |
| <i>Perfluorocarbons</i> | |
| Large-scale production | Need to be emulsified |
| Low production costs | Particles not uniform size |
| Prolonged shelf life | High FiO ₂ required |
| Minimal infection risk | Low O ₂ capacity at physiological O ₂ |
| Minimal antigenicity | Rapid plasma clearance |

FIRST GENERATION HEMOGLOBIN SOLUTIONS Some hemoglobin solutions were initially developed by military or academic institutions, but all are now licensed to commercial companies for development (Table 91-2). They have most commonly used outdated human blood donations as the source of hemoglobin, although one company (Biopure) uses bovine blood, whereas another (Somatogen) used recombinant technology. The biological challenges to the development of a hemoglobin solution include the following.

1. Free hemoglobin dissociates into α/β dimers that oxidize rapidly and are cleared by the kidney, resulting in a short half-life and in renal toxicity if their concentration reaches critical levels. Various mechanisms have therefore been used to crosslink the α/β -subunits through either the α - or β -chains, using chemicals or recombinant technology.
2. In the absence of 2,3 DPG, the oxygen dissociation curve of free hemoglobin is left shifted when compared to hemoglobin in red cells. Early investigators considered this as a potentially useful characteristic, but this increase in oxygen affinity means that oxygen does not readily off-load from the carrier to the tissues. Strategies taken to reverse this shift include pyridoxylation, site-directed mutagenesis, polymerization, and internal crosslinking, all discussed later. A completely different approach is to use bovine hemoglobin, which, although having 90% sequence homology with human hemoglobin, responds allosterically to chloride ion rather than to 2,3 DPG. Therefore, at physiological chloride concentrations, its oxyhemoglobin dissociation curve resembles that of human hemoglobin within red cells.
3. Free hemoglobin exerts considerable oncotic pressure, drawing fluid from the tissues into the circulation.

A variety of modifications were therefore introduced into the molecule by different companies to counteract the problems previously outlined (Fig. 91-1).

Chemical Crosslinking of α - or β -Chains This not only prevents dimerization *in vivo* but in theory could stabilize the molecule sufficiently to permit viral inactivation. The HemAssist product developed by Baxter used 3,5 *bis* (dibromosalicyl) fumarate to crosslink the α -chains. The dimensions of the crosslinker meant that it reacted only with Lys α_1 99 and Lys α_2 99 in the internal cavity of

the molecule, binding the two halves together. This also had the useful effect of raising the P₅₀ (Fig. 91-2), but the product had significant vasoactivity in animal studies of shock. Phase III clinical trials in stroke and trauma gave disappointing results, and development of the product was discontinued in 1998.

The Hemolink product developed by Haemosol in Canada uses open ring (oxidized) raffinose to crosslink specific amino acids in the β -chains in the 2,3 DPG pocket, which also has the effect of raising the P₅₀. The *O*-raffinose is also used to polymerize the molecule.

Polymerization Polymerization can be achieved through the use of crosslinkers that target surface amino groups. Polymers of various lengths can be produced using glutaraldehyde, as the reaction can be quenched using lysine. This strategy has been employed by Northfield Laboratories to polymerize human hemoglobin (PolyHeme). This product is also pyridoxylated, which raises the P₅₀, resulting in a product similar to human blood in terms of oxygen affinity and colloid oncotic pressure. Glutaraldehyde polymerization is also used by Biopure for production of their bovine hemoglobin product (Hemopure). This product can transport oxygen to the tissues effectively, and even in low doses can raise arterial pressure and lower cardiac index. It was reported to be life saving in a case of severe autoimmune hemolytic anemia, and has been licensed in South Africa for orthopedic surgery. Currently, a trial is proposed in which Hemopure is used in cardiac surgery to improve tissue oxygenation by diffusion through an atheromatous obstruction in coronary arteries. Finally, as mentioned, the HemoLink product is polymerized using *O*-raffinose.

Recombinant Technology Building on ideas generated at the UK's Medical Research Council laboratories in Cambridge, Somatogen (and later Baxter Healthcare) developed a human hemoglobin solution produced in *Escherichia coli*, using synthetic genes encoding the human α - and β -globin polypeptides and expressed from a single operon. The purified recombinant hemoglobin had the correct stoichiometry of the α - and β -globin polypeptides, which associated into soluble tetramers, and incorporated heme. Each globin chain also contained an additional methionine as an extension to the amino terminus. The recombinant hemoglobin had a high-performance liquid chromatography profile essentially identical to that of human hemoglobin A and comigrated with hemoglobin A on polysaccharide gel electrophoresis. The visible spectrum and oxygen affinity were similar to that of native human hemoglobin A, but the recombinant protein showed a reduction in Bohr and phosphate effects, possibly because of the presence of the methionine at the amino termini of the α - and β -chains.

Because the oxygen affinity of this free hemoglobin in the absence of 2,3 DPG was too high to allow adequate oxygen offloading in the tissues, a variant was created using an expression vector containing one gene encoding the naturally occurring Asn-108 β to Lys mutation (hemoglobin Presbyterian), which reduces the oxygen affinity and enhances the Bohr effect slightly. In addition, to prevent $\alpha\beta$ -dimerization and renal toxicity, and to increase the half-life, the product was made using one duplicated, tandemly fused α -globin gene, resulting in end-to-end fusion of the two α -globin-subunits. The short distance (2–6 Å) between the N-terminal residue of one α -chain and the C-terminal residue of the other allowed the insertion of a glycine residue as a bridge. Despite these elegant modifications, this product led to gastrointestinal side effects, and Baxter discontinued its development at the same time as studies on HemAssist were stopped.

Table 91-2
Red Cell Substitutes and Their Current Status

| Company | Product | Source | Modification | Development status |
|-------------------------------------|---------------------------------------|----------------------|--|--|
| <i>Perfluorocarbons</i> | | | | |
| Alliance Pharmaceutical Corporation | Oxygent | — | Emulsified perflubron | Phase III cardiac surgery, hemodilution, hemorrhage |
| Hemogen | Oxyfluor | — | — | Discontinued |
| <i>Hemoglobin solutions</i> | | | | |
| Apex Bioscience (now CuracYTE) | PHP | Human | Pyridoxylated; conjugated to polyoxyethylene, binds NO | Phase IIc, shock |
| Baxter Healthcare | HemAssist | Human | α - α crosslinked with “diaspirin” | Discontinued 1998 at phase III stage |
| Biopure | Hemopure | Cow | Glutaraldehyde-polymerized; purified to remove tetramers | Phase III with hemodilution, +compassionate use Licensed for surgery in South Africa FDA license submitted Trial planned in cardiac surgery |
| Biopure | Oxyglobin | Cow | Glutaraldehyde-polymerized | Licensed for use in dogs |
| Enzon | PEG-hemoglobin | Cow | PEG; tetramer | Not in active development |
| Hemosol | Hemolink | Human | O- <i>raffinose</i> to crosslink β -chains and to polymerize; tetramers remain | Phase III cardiac surgery, studies with erythropoietin in renal failure and surgery |
| Northfield Laboratories | PolyHeme | Human | Pyridoxylated; glutaraldehyde polymerized; purified to remove tetramers | Phase III trauma |
| Sangart | Hemospan (MP4) | Human | PEG; polymer; virus treated with riboflavin | Phase II, orthopedics, Sweden, and now USA |
| Somatogen/Baxter | Hemospan PS Optro-r hemoglobin-1.1 | Human Recombinant | Hemospan + pentastarch Tetramer; α -chains fused end to end; β -chain mutation | Discontinued 1998 |
| Synzyme | Hemozyme | Human | Polymerized | Preclinical |

FDA, Food and Drug Administration; PEG, polyethylene glycol; PHP, pyridoxal hemoglobin polyoxyethylene.

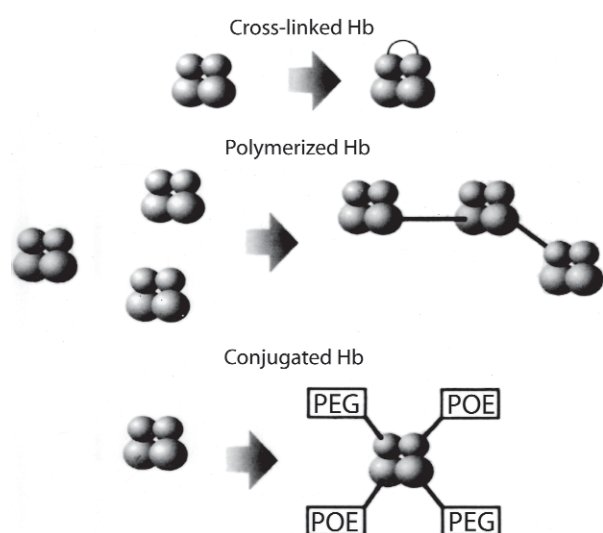


Figure 91-1 Internally crosslinked, polymerized, and surface-modified hemoglobins. (Reprinted with permission from Blackwell Publishing from Stowell CP, Levin J, Spiess BD, Winslow RM. Progress in the use of blood substitutes. *Transfusion* 2001;41[2]:287–299.)

Surface Modification The 42 lysine amino groups on the surface of hemoglobin are targets for attachment of small molecules such as polyethylene glycol (PEG) and polyoxyethylene (POE), which stabilize the tetramer and increase the molecular

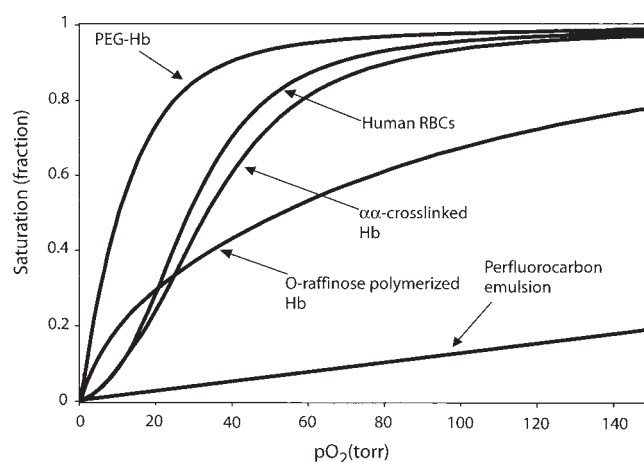


Figure 91-2 Oxygen dissociation curves of various hemoglobins and perfluorocarbon. Note that all hemoglobins give sigmoid curves, whereas perfluorocarbon gives a linear relationship. (Reprinted with permission from Blackwell Publishing from Stowell CP, Levin J, Spiess BD, Winslow RM. Progress in the use of blood substitutes. *Transfusion* 2001;41[2]:287–299.)

weight. The resulting increase in oncotic pressure and viscosity was considered therapeutically useful in expanding the plasma volume. It was also thought that this type of modification would render the molecule less visible to the reticulo-endothelial system, minimizing its antigenicity. Products in this category include

bovine hemoglobin modified by PEG, which was manufactured by Enzon, but this may no longer be in active development. However, a human hemoglobin solution designated pyridoxal hemoglobin polyoxyethylene developed by Apex Bioscience is in phase IIc trial in shock.

Encapsulation To prevent the renal toxicity and short half-life associated with free hemoglobin, attempts have been made to enclose hemoglobin in liposomes consisting of synthetic lipids in various combinations. Phosphatidylcholine has been used to produce bilamellar spheres loaded with a hemoglobin solution, whereas the addition of cholesterol increases elasticity and half-life. In theory, the oxygen affinity could be modulated by the inclusion of 2,3 DPG, whereas methemoglobin reductase could be added to prevent oxidation. However, there are technical difficulties in controlling the size of the liposomes, and it is not yet clear what effects the clearance of large amounts of phosphatidylcholine would have on the reticuloendothelial system. These products remain at the preclinical stage of development.

PROBLEMS WITH FIRST GENERATION OF SUBSTITUTES AND NEW APPROACH TO HEMOGLOBIN SUBSTITUTES Many first generation compounds failed clinical trial because of unacceptable hypertension, bradycardia, chest and abdominal pain and nausea/vomiting. At first these effects were thought to be owing to contaminants, but are now considered to be the result of reflex vasoconstriction. Discoveries regarding the functioning of the microvasculature, facilitated by new techniques for viewing small vessels, have changed the thinking behind the ideal properties of a hemoglobin substitute.

1. Identification of NO as the endothelium-derived relaxing factor. The heme group binds NO with high affinity, and this is especially true of free hemoglobin. This property of free hemoglobin contributes to the hypertensive properties of some solutions.
2. The realization that diffusion of cell-free oxygenated hemoglobin can be harmful, as it leads to constriction of precapillary arterioles and thus reduces tissue perfusion. Significant amounts of oxygen leave the circulation at the precapillary arteriolar stage. These vessels are rich in nerve endings, and appear to be extremely sensitive to oxygen. The so-called facilitated diffusion of oxygen that is bound to the free hemoglobin can be limited by increasing both the diameter of the hemoglobin molecule itself and the viscosity of the solution. Modified hemoglobin molecules of different radii have varying vasoactive properties, even though their interaction with NO is the same. Widespread prearteriolar vasoconstriction in the presence of a normal blood volume leads to hypertension; conversely vasoconstriction with an inadequate blood volume results in shock, volume depletion and ultimately collapse of the nonmuscular capillary walls, which rely on vascular pressure to keep them open. To overcome this undesirable side effect, oxygen availability from the hemoglobin substitute should be less than that from natural red cells. Oxygen availability to the precapillary arterioles can be limited by increasing the oxygen affinity of the molecule and decreasing its concentration in the solution.
3. Production of vasodilators stimulated by shear forces on the endothelium can be maintained by having a solution of fairly high viscosity. At rest, therefore (though not during exercise), the maintenance of microvascular blood flow, vascular volume and acid-base balance are more important than

Table 91-3
Past and Current Concepts in the Design of a Blood Substitute

| <i>Parameter</i> | <i>Considered optimal for first generation oxygen carriers</i> | <i>Considered optimal for second generation oxygen carriers</i> |
|--------------------------|--|---|
| Oxygen binding | Like blood: P50 28 mmHg | P50 5–10 mmHg |
| Viscosity | Like water: 1 cP | Like blood: 4 cP |
| Oncotic pressure | Like blood: 15 mmHg | Increased |
| Hemoglobin concentration | Like blood: 15 g/dL | As low as possible |

Modified from Winslow RM. Current status of blood substitute research: toward a new paradigm. *J Intern Med* 2003;253(5):508–517.

oxygen carrying capacity. These observations change the theoretical requirements of an oxygen carrier (Table 91-3). This logic has determined the design of the modified hemoglobin solution Hemospan (MP4, Sangart, see Table 91-2).

Made from outdated red cells, this compound has the lysine residues thiolated then further surface modified by the addition of PEG. It is subjected to pathogen reduction using the riboflavin technique. This solution has a P_{50} of only 4 mmHg, which is predicted to limit early oxygen off-loading at the arteriolar stage. The large increase in radius as a result of the PEG also limits the undesirable oxygen diffusion, maintains plasma viscosity, and hence shear forces on the endothelium, and confers a half-life of 43 h. The compound resulted in 100% survival and absence of hypertension in a hemorrhagic rat model (Fig. 91-3), and in primate studies, no vasoconstriction or hypertension was seen. The compound is now in phase II clinical trial in orthopedic surgery in Sweden, and a trial in the United States is planned.

PERFLUOROCARBONS The perfluorocarbon class of compounds is characterized by a linear or cyclic carbon backbone that is highly fluorinated, with other halogens sometimes also present.

They can dissolve large amounts of oxygen and CO₂ whereas being chemically and biologically inert. Their properties were famously displayed in images of a mouse living and breathing “underwater” in a beakerful of oxygenated fluorocarbon. At a pO_2 of 100 torr, dissolved oxygen can reach 10 mL/dL, compared with 0.5 mL/dL for plasma and 20 mL/dL in blood with a hemoglobin concentration of 15 g/dL. Unlike hemoglobin, the oxygen dissociation curve is linear (see Fig. 91-2), so if the patient can breathe very high levels of inspired oxygen, greater amounts of oxygen can be carried. However, because fluorocarbons are immiscible with plasma, they have to be emulsified by mixing with a surfactant (usually a phospholipid) to produce particles of submicron size, although precise size is difficult to control. The surfactant can comprise a high percentage of the solution by weight, and may not be totally biologically inert.

The original compound, Fluosol-DA, was licensed by the US Food and Drug Administration in 1990 for use in percutaneous transluminal coronary angioplasty, but became obsolete and was withdrawn for this purpose in 1994. Clinical trials in acute hemorrhage demonstrated no improvement in clinical outcome. Later compounds have smaller particle size, which should reduce flu-like symptoms attributable to cytokine and complement activation, and greater solubility for oxygen. Oxygent (Alliance Pharmaceuticals) is an emulsion of perflubron (perfluoro-octyl bromide) and egg yolk phospholipid. It is in a phase II/III clinical trial to assess

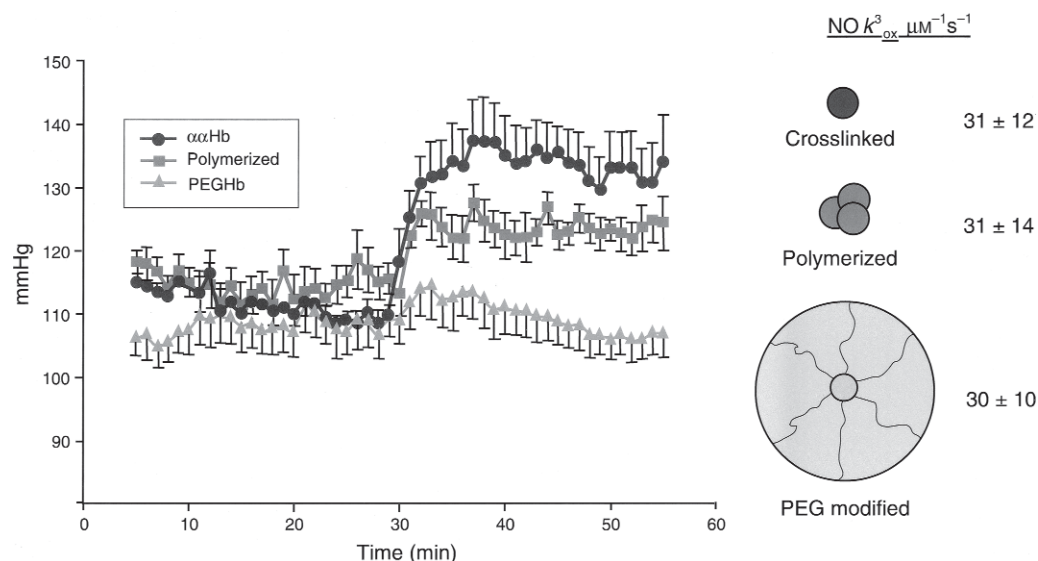


Figure 91-3 Mean arterial pressure response in the rat to infusion of three modified hemoglobins. The sizes of the molecules are drawn to scale relative to each other, and the association rate constants for NO are given at the right. (Reprinted with permission from Blackwell Publishing from Winslow RM. Current status of blood substitute research: toward a new paradigm. *J Intern Med* 2003;253[5]:508–517.)

whether its use can increase the permitted degree of acute normovolemic hemodilution, thus improving the extent of blood saving. A further trial is designed to examine its effect in acute hemorrhage. Another fluorocarbon (Oxyfluor) was designed to absorb the microbubbles that form on cardiopulmonary bypass circuits and are thought to be responsible for neurovascular complications. This is no longer in development.

ONGOING ISSUES IN THE DEVELOPMENT OF A BLOOD SUBSTITUTE

How to Evaluate the Product Regulatory authorities increasingly expect clinical end points from studies of new products. One of the difficulties is that the red cells used as controls have not themselves been subjected to studies of similar rigor, and there are no established criteria for assessing their efficacy. Mortality and transfusion avoidance have been used in some trials, but other end points may evolve as different clinical scenarios are studied.

Short Half-Life Short half-life is still a major problem. Even the best substitutes (encapsulated/surface modified) have circulatory half-lives of only 2–3 d, with most being only 12–24 h. This still restricts the usage to acute hemorrhage, with no sign of a product to treat chronic anemias.

A Safe and Reliable Source of Start Material Although not an issue for perfluorocarbons, of which there is limitless supply, sourcing adequate amounts of human hemoglobin is a challenge. With optimal stock management, outdates are low (<2% in England), and for safety reasons, the donor exclusion criteria cannot be relaxed to increase supply. Yields of free hemoglobin from outdated red cells are rarely more than 50%, so targeted blood collections would have to be organized to provide start material.

The use of bovine blood seems questionable at this point, as possible prion transmission is a key concern of blood suppliers. Accredited bovine spongiform encephalopathy-free herds could be developed, but the possibility of unknown bovine agents exists. Recombinant technology remains a possibility once the optimal formulation is established, but it is uncertain whether any process could be adequately scaled up to meet demand.

The Safety/Cost Balance In the two decades of development of blood substitutes, blood itself has become increasingly safe from the virus point of view. The residual risk of HIV, HBV, and HCV is now 1 in 10^{5-6} /U in many parts of the world. Even when a new agent emerged (WNV in the United States and Canada in 2002), genome testing was in place within 12 mo. Developed countries are questioning the spiraling costs of blood components, and the high costs of steps providing only small incremental increases in safety. In the United States, virus inactivated pooled solvent detergent fresh frozen plasma (FFP) did not become an established product, and is now withdrawn. In Europe, in which one pathogen inactivation system is now licensed for platelets, uptake is limited, and cost-effectiveness is one factor in this. Ironically, the parts of the world that might gain most benefit from a blood substitute are unlikely to be able to afford it. Another related issue is the safety of the products themselves. As blood safety increases, the acceptance of possible serious side effects of a blood substitute will fall.

Further Advances in Knowledge of the Red Cell Intriguingly, a recent study suggested that red cells themselves may be able to respond to hypoxia by releasing NO generated from the reduction of nitrite by deoxyhemoglobin. It remains to be seen whether this newly discovered property of red blood cells will present another obstacle in the search for an artificial oxygen carrier.

SUMMARY Although it might be considered disappointing to not have a single licensed blood substitute, a great deal has been learned about the physiology of oxygen delivery to tissues and the sequence of events when it goes wrong. The next decade may see one or more products reach the clinic. Issues of cost-effectiveness, safety, and availability will ultimately determine whether these products become true blood substitutes or niche products for a limited number of patients.

GENOMIC DETECTION OF VIRAL AGENTS

METHODS Methods enabling the detection of the genome of infectious agents have been developed, initially in research, then for diagnostics, and finally for blood screening. Methods such as direct hybridization and its more sensitive version called “branched

Table 91-4
Impact of HCV and HIV NAT on Blood Safety

| Country | Author | Number screened (millions) | Estimated risk/million transfusions | | | |
|---------------|----------|----------------------------------|-------------------------------------|----------|---------|----------|
| | | | HCV | | HIV | |
| | | | Pre NAT | Post NAT | Pre NAT | Post NAT |
| United States | Stramer | 35.7 | 4.3 | 0.61 | 0.75 | 0.52 |
| France | Pillonel | 4.9 | 1.0 | 0.15 | 0.71 | 0.4 |

HCV, hepatitis C virus; NAT, nucleic acid testing.

DNA” are not adapted to the mass screening approach required for testing in the transfusion context. Methods including amplification such as PCR, ligase chain reaction, transcription mediated amplification, or nucleic acid sequence-based amplification have been developed and used in transfusion, in which requirements of high sensitivity, high specificity, and high throughput need to be met. Quantitative PCR based on the use of Taqman probes has been introduced and seems to be well adapted to the demand of the transfusion market.

Nucleic acid testing (NAT) was first introduced in 2000 for the detection of the hepatitis C virus (HCV) genome. Soon after other NAT targets such as HIV, hepatitis B virus (HBV), and WNV were added, creating a momentum for the development of multiplex NAT, enabling simultaneous detection of genomes of two (duplex) or three (triplex) different viruses in a single reaction tube.

Automation is critical in blood screening for transfusion. No system is available to integrate all three basic steps of NAT: nucleic acid extraction, amplification, and detection, in a unique automated instrument. A fair amount of manual operation remains that will be eliminated when the prototype automated instruments become commercially available.

SAMPLES The considerable cost of NAT on a large scale led to the development of the concept of plasma pool testing by which mixtures of individual plasma samples from blood or plasma donations are mixed and tested in a pool. The pool size ranges from 8 to 512 depending on the target genome and the method used. Pooling has a clear impact on test sensitivity. This inherent lack of sensitivity was overcome in different ways: one was extreme sensitivity of assays with the attached toll on specificity, the other was concentration of targets by various methods, the most popular being ultracentrifugation. When NAT targeted viruses such as HCV or HIV that replicate very rapidly to viral load above 10^5 copies/mL, pooling did not substantially affect screening efficacy, although most national blood transfusion organizations progressively reduced the number of samples per pool from 96 to 48, 24, and 16. The lower viral load observed with HBV and WNV during the infectious window period led blood services to reduce further pool size to 8 or 10 samples. Ultimately, individual donation NAT already implemented by some blood organizations will likely become the rule, particularly with the relative decrease in costs introduced by multiplexing.

IMPLEMENTATION OF NAT IN TRANSFUSION NAT was first introduced in the late 1990s by the plasma derivative industry following the transmission of HCV by intravenous IgG that was not virally inactivated. NAT of various sensitivities was applied to large pools of plasmas prepared for fractionation. Later, despite the initial reticence of both regulators and the blood bank community, NAT applied to HCV RNA was mandated by the EU and US regulatory bodies but the pool size was not specified, only a clinical sensitivity of 5000 IU/mL was to be achieved by the methods used. In 2004, all EU countries have implemented HCV NAT. HIV NAT

was simultaneously implemented in France, Germany, and the United States, soon followed by other countries such as Australia, Japan, and more European countries.

The WNV epidemics in the United States that spread between 1999 and 2003 raised a new level of concern regarding transmissibility of emerging viruses by transfusion, and in a 6-mo period, commercial NATs under investigational new drug regulation were implemented in July 2003 in the United States and Canada. The epidemiology of WNV in other areas such as Western Europe does not warrant a similar approach.

Discussions are ongoing to determine the usefulness of NAT for other transfusion transmissible viruses with known residual risk such as HBV and human erythroviruses that include parvovirus B19. HBV DNA screening is already implemented in Japan, Germany, Austria, and Luxembourg. The successive implementation of several NATs in addition to the serological screening assays is adding not only to the financial burden, but also to the complication of operations, therefore increasing the risk of errors. Following implementation of HCV, HIV, and HBV NAT, the rate of errors has become a major factor in calculating residual risk of viral transmission by transfusion. Complete automation is expected to reduce that risk. However, as the number of NATs is likely to continuously increase, alternative strategies such as pathogen inactivation need to be considered, both from a safety and a financial point of view.

IMPACT OF NAT ON BLOOD SAFETY The impact of NAT on the safety of the blood supply has been estimated in several countries. Owing to the rarity of transmission cases, no direct assessment can be done and only calculated estimates can be provided. Table 91-4 indicates preliminary results from France and the United States.

In the present circumstances of a widespread epidemic, epidemiological review of the WNV NAT implemented in North America revealed that several hundred viremic donations had tested positive, and it is estimated that approx 1000 cases per year of transmission would be avoided. This result contrasts with the 5 and 55 cases avoided per year with the HIV and HCV NAT, respectively.

Emerging viral infections originating either from new viruses (mostly animal) breaking into humans or from previously unknown viruses recently discovered are candidate for additional NAT. However, before implementation, several criteria need to be met. The emerging virus should be pathogenic, transmissible by transfusion, in relatively low prevalence, and infectious/persistent for a long enough period of time. The recent severe acute respiratory system, SARS, infection epidemic falls into the first category; although no evidence of transfusion transmission has been provided. The second category includes GB virus-C, TT virus, and SEN virus. The first virus is of low prevalence but no evidence of pathogenicity has been found, whereas the other two, which are in the same family of human circoviridae, are ubiquitous and with no identified pathogenicity.

MOLECULAR DETECTION OF BACTERIALLY INFECTED BLOOD Bacterial infection of blood components is relatively frequent in platelet concentrates because this product is stored at 20°C under constant agitation, which is highly favorable to growth of aerobic bacteria. Among the many methods developed to detect bacterial contamination, the most effective practiced method is culture, with detection either by accumulation of CO₂ or consumption of O₂. These methods are very sensitive, but require a period of natural bacterial growth to amplify the number of bacteria to a detectable level. This procedure takes time (at least 24 h), a major handicap for a product whose shelf life is only 5 d. Molecular methods including an amplification step can overcome this drawback but the diversity of bacterial genome makes it difficult to cover a large spectrum of organisms. Methods targeting the 16S ribosomal (r)RNA have been proposed, whereas others use a universal bacterial rRNA probe. To date, despite a sensitivity of 6–500 organisms/100 µL, these methods do not cover a sufficiently large spectrum of bacteria and are considered expensive and labor intensive. Considerable development is needed before molecular methods can be considered as suitable replacement for methods involving culture.

MOLECULAR DETECTION OF PARASITES IN TRANSFUSION MEDICINE Parasites are transmissible by transfusion but are mostly limited to circumscribed geographic areas. However, immigration from endemic areas and intensive travel have resulted in a real risk of malarial transmission from African and Asian tropical areas in the Northern hemisphere and of *Trypanosoma cruzi* transmission from Central and South America to the Northern part of the American continent. Rules of donor exclusion according to place of origin or traveling have been implemented, but the yield is poor and the number of undeserved donor deferrals high. Various methods have been proposed to distinguish between donors presenting with and without a real risk of transmission. In the case of malaria, antibody detection identifies individuals who have been infected in the past but are not necessarily infectious, whereas antigen detection or thick blood smear are not sufficiently sensitive and, for the latter, discouragingly labor intensive. Genomic amplification methods have therefore been developed using nested PCR or quantitative PCR to detect one parasite per 100 µL of whole blood. The cost-effectiveness of such methods has been evaluated but their clinical efficacy remains to be assessed.

T. cruzi infection is highly immunogenic, with infection occurring mostly in young children. The presence of antibodies in a blood donation is considered synonymous with infectious risk, and high-performance assays are systematically used in endemic areas. Molecular methods have not been considered to help in the deferral of at risk blood donors.

A similar situation is found with tick-transmitted parasites such as *Babesia* that is endemic in the Northern hemisphere.

PATHOGEN REDUCTION OF BLOOD COMPONENTS

Although they have resulted in extensive reductions in risk, donor selection and testing have not eliminated pathogen transmission by transfusion. With the continual discovery of new pathogens, it is clear that the addition of a new test for each adds complexity and loss of donations and donors with false positive reactions. Pathogen inactivation is an adjunct and in some cases a possible alternative to developing individual tests and donor exclusion criteria to address each pathogen individually.

Since the early 1990s, a long series of compounds have been evaluated for their ability to inactivate viruses or other infectious agents without unacceptable loss of cellular function. The last and

Table 91-5
Pathogen Inactivation Methods for Blood Components

| <i>Component</i> | <i>Compound</i> | <i>Phase of development</i> |
|------------------|--------------------|---|
| Platelet | Amotosalen (S59) | Clinical practice EU |
| RBC | FRALE (S303) | Interrupted |
| RBC | Inactine (PEN 110) | Interrupted |
| FFP | Amotosalen (S59) | Phase 3 completed; submitted for EU license |
| FFP | Solvent detergent | Clinical practice EU, discontinued in United States |
| FFP | Methylene blue | Clinical practice EU |

EU, European Union; FFP, fresh frozen plasma; RBC, red blood cell.

most promising generation of such compounds targets nucleic acids directly, which are altered and made unable to replicate. In addition to having the potential to inactivate viral and bacterial nucleic acids, compounds such as Psoralens (S59), Inactine, and Riboflavin can inactivate parasites and leucocytes, particularly lymphocytes responsible for transfusion-associated graft-vs-host disease, a universally fatal complication. These follow other chemicals such as methylene blue or solvent detergent-based methods already in use. Because solvent/detergent is effective by disrupting viral (and possibly bacterial) membranes, the spectrum of activity is limited to enveloped viruses.

Among the compounds listed in Table 91-5, Solvent detergent and methylene blue can only be used in fresh frozen plasma as it disrupts cell membranes. Clinical use of Amotosalen has been started in some European countries, following CE marking. Published randomized trial results indicate efficacy of the system, and although there is a systematic reduction of platelet yield compared to standard products, this difference did not seem to affect clinical efficacy in preventing bleeding. In vitro and in animals, no evidence of oncogenic or other toxicities have been found in short- and long-term studies. However, clinical trials of two different pathogen reduction systems for red cells (S303 and Inactine) have both been suspended following development of red cell alloantibodies. The long-term future of pathogen reduction of red cells and platelets remains to be seen.

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