Introduction

Seventy percent of severe hemophiliacs in the United States have been infected with human immunodeficiency virus (HIV), which contaminated the coagulation factor concentrates they used in their treatment largely between the years 1979 and 1985. World-wide, most severe hemophiliacs are infected with hepatitis B virus (HBV) and/or hepatitis C virus (HCV). In the early 1970's in the United States, approximately 10% of recipients of single donor blood products, i.e. Whole Blood, Red Blood Cell Concentrates (RBCC), Platelet Concentrates (PC), and Fresh Frozen Plasma (FFP), inadvertently were exposed to HBV or HCV. In the early 1980's, one in 10,000 recipients of these same products was exposed to HIV.

The emergence of HIV in the general population served to mobilize action at all levels of government, industry and academia. Through scientific inquiry and understanding, creativity, and perseverance, each of the risks described above has been reduced substantially. Coagulation factor concentrates prepared from plasma pools no longer transmit HIV and seldom transmit HBV or HCV. The risk of HIV, HBV, or HCV exposure from a single donor blood product is estimated at 1/225,000, 1/200,000, and 1/3,300 to 1/6,000, respectively, per unit infused. Had those of us charged with the safety and provision of blood to hospitals and physicians not responded adequately to the presence of HIV in donor blood, our industry would be decimated today. To the extent we were slow to respond, or perceived to have responded slowly, we suffer the consequences. It is the perception amongst many that it did not have to happen, and that inaction was the result of an insular community and, in some cases, avarice. It is the perception that government was a co-conspirator of inaction. Importantly, the public perceives that the dangers of the 1980's continue today. Since patient concern has never been higher, the medical community must know the facts as distinguished from the perceptions and must act to regain the public's confidence.

Over the past decade, blood banking and blood processing procedures and the practice of transfusion medicine have changed substantially. Today, we are more aware of the dangers of blood transfusion and of steps to reduce if not eliminate these dangers. Blood donors are examined and questioned more closely than ever before in an attempt to eliminate donors who are more likely to harbor an infectious, blood-borne virus. Every donation is tested by new and more sensitive blood tests, and in some cases, blood
screening tests are introduced even before benefit is established. Donor histories and test results have been computerized, and the error prone manual transcription of critical information is being eliminated. Manufactured blood products are more highly purified than ever before, and purification procedures have been modified to more consistently reduce viral load. Virus inactivation technology is in wide-spread use in the preparation of coagulation factor concentrates, and validated virus inactivation methods are beginning to be applied to all blood protein solutions including immune globulins and FFP. One could not fathom introduction of a new blood protein product today if it was not virally inactivated. Regulatory oversight has increased and standards common in the pharmaceutical industry are beginning to be applied to blood. Where feasible, autologous transfusions have replaced homologous, and to eliminate unnecessary blood exposure, hospital use committees regularly examine transfusion practice.

While it has and continues to be a challenging and difficult time, we each have much to be proud of and can take some comfort in the statement that "blood has never been safer".

Nonetheless, our work needs to continue. The public has demanded the safest possible blood supply and we should do everything in our power to achieve it. To do otherwise will result in continued criticism, law suits, and inquiries into blood banking practices. Moreover, patient distrust can lead to donor distrust, eroding the underpinning of voluntary blood donor systems.

With respect to viral safety, the data are clear -- the only way to achieve absolute safety is through viral inactivation, and numerous advantages accrue on adoption of virus inactivation processes. The window period will no longer be of concern, errors in testing or the inadvertent release of a blood unit which tests positive no longer will result in viral transmission, new viruses or new viral serotypes will be eliminated even before their presence is recognized, and tests for rare viruses need not be deployed.

Viral Inactivation of Coagulation Factor Concentrates

Nowhere can the value of virus inactivation be illustrated better than in the preparation of coagulation factor concentrates. Antihemophilic factor concentrate (AHF) and prothrombin complex concentrate manufactured without viral inactivation transmitted HIV, HBV, and HCV with high frequency. As late as 1985, essentially every vial of these concentrates were contaminated by HCV. With the advent of viral inactivation, HIV transmission was virtually eliminated. For example, in the United States there has not been a single documented case of HIV transmission associated with concentrate infusion since 1987. While some of the first methods of viral inactivation failed to eliminate HBV and HCV transmission, currently employed methods have excellent records of safety also with respect to these viruses. Focussing on carefully constructed clinical studies in previously untreated patients, hepatitis has not been transmitted by products which have been pasteurized, vapor heated, dry heated at 80°C for 72 hours, or treated with
solvent/detergent. Substantial community use information supports a conclusion of safety. For example, with solvent/detergent treated products, used in the preparation of approximately two thirds of the world's plasma derived coagulation factor concentrates, over 6 billion units have been infused without a single documented case of HIV, HBV, or HCV transmission. While several reports indicate transmission of HBV or HCV with pasteurized or vapor heated products, reports of transmission are rare and may be consistent with community and not product sources of virus. Based on probability determinations where viral load is compared with documented viral elimination, modern AHF concentrates prepared with solvent/detergent have a high probability of safety, calculated as less than one infectious unit of HIV, HBV, or HCV per 10^{18}, 10^{12}, and 10^8 vials of product, respectively.

Each viral inactivation method in common use today has distinctive features. Solvent/detergent treatment can be applied equally and predictably to complex protein mixtures such as plasma and to highly purified protein solutions. Because action is directed toward lipids, enveloped viruses are inactivated rapidly and completely and non-enveloped viruses and proteins (except for lipoproteins) are unaffected. Methods of thermal inactivation are advantageous in that all classes of virus are potentially susceptible, although non-enveloped viruses tend to be heat stable. Because thermal inactivation methods are not inherently specific, means of stabilizing proteins while achieving excellent virus inactivation had to be identified. With pasteurization, proteins are stabilized by addition of high concentrations of low molecular weight solutes, especially sugars and amino acids. Although viruses are also stabilized, relatively good discrimination can be achieved, although at some cost in protein recovery. With dry heat, proteins are stabilized by reducing moisture content and process recovery can be high. Unfortunately, lyophilized cakes are heterogeneous with respect to solute and moisture content both within the vial and between vials. Reproducible viral safety required that products be reformulated, use of 80°C in place of 60°C, and with vapor heating, introduction of additional moisture. One advantage of the dry heat method is that it can be performed on product in the final container, eliminating the possibility of post-treatment re-contamination. With all other methods, re-contamination is prevented by separating pre- and post-virus inactivation areas, equipment, and personnel.

Despite the sharp reduction and possibly elimination in transmission of HIV, HBV, and HCV, the apparent transmission of hepatitis A virus (HAV) by an ion exchange purified, solvent/detergent treated AHF concentrate in several European countries raised concerns about non-enveloped viruses, first because they are not inactivated by solvent/detergent treatment and second because they tend to be heat stable. Viruses in this class include HAV and parvovirus B19. Consequently, manufacturers are examining the use of two complementary viral elimination methods. The advantage of combining methods which act by independent mechanisms is that both a broader spectrum and a higher quantity of viruses can be eliminated. As examples, antibody affinity purification validated as a virus removal method has been combined with either solvent/detergent or heat treatment; some products are now treated by both solvent/detergent and heat; other
products have been processed through viral removal filters which have been developed recently and added to existing processes. New methods of virus inactivation under exploration include use of chaotropes such as sodium thiocyanate, short wavelength ultraviolet light in the presence of antioxidants, microwave heating, extraction with supercritical fluids, iodine, and light activated compounds such as psoralens and methylene blue. Given the extensive history of safety with respect to the principal viruses of concern achieved by currently employed methods and the limitations of laboratory and pre-clinical virus validation studies, the addition to rather than the replacement of existing processes seems likely. As an example, research at the New York Blood Center has shown that combining solvent/detergent with UVC irradiation in the presence of an antioxidant in the treatment of an AHF concentrate results in the kill of a wide variety of viruses, including HBV, HCV, HIV, HAV, and parvovirus. Similar results were obtained when solvent/detergent was combined with filtration using a filter with 15 nm pore size. Unfortunately, 35 nm filters do not block passage of HAV and 15 nm filters cannot be used efficiently with large protein molecules or high protein concentration.

Other Purified Blood Protein Derivatives

The success in applying viral inactivation methods to coagulation factor concentrates raises the question as to whether all blood protein products should be virally inactivated. This question is especially pertinent to immune globulin solutions which generally have a history of safety without use of a specific virus inactivation method. Recently, one intravenous immune globulin preparation was recalled world-wide because of a clear association with HCV transmission to over 100 recipients. Careful study of the fractionation characteristics of HCV suggests that this outbreak was caused by the recent adoption of generation 2 HCV antibody screening since the reduced level of anti-HCV antibody in the plasma pool resulted in more virus co-isolating with IgG during cold ethanol fractionation. It also seems probable that the lower titer of anti-HCV antibody in the final product reduced immune neutralization. Consequently, the United States FDA has recommended that all immune globulin preparations, including intramuscular, intravenous and hyperimmune gamma-globulins, be virally inactivated. Incorporation of a defined viral inactivation procedure into a manufacturing scheme will provide greater freedom when plasma collection or testing procedures or purification processes are modified in the future.

Viral Inactivation of Fresh Frozen Plasma

At one time, the viral risk of coagulation factor concentrates greatly exceeded that from FFP. Because of viral inactivation, products prepared from plasma pools are now safer than the individual units from which they were derived. This change in safety profile encouraged the development of solvent/detergent-treated plasma as a substitute for FFP. Virus inactivation has been validated extensively, including studies demonstrating the complete inactivation of added HIV, HBV and HCV. Coagulation factor content is similar to that found in the start plasma, and, because of plasma pooling, all lots contain high concentrations of anti-HAV and anti-parvovirus antibodies. Since these antibodies are
known to be neutralizing and protective, the likelihood of transmission of either virus seems remote. Clinical trials confirm efficacy and safety in the treatment of coagulation factor deficits where concentrates are either inappropriate or unavailable and in the treatment of thrombotic thrombocytopenic purpura. SD-Plasma is now routinely used in France, Germany, Norway, and Austria and a Product License is pending in the United States. More recently, pasteurized pooled plasma and single donor plasma treated with methylene blue and visible light have been developed. Formal studies on the inactivation of HBV or HCV are pending. Pasteurized plasma is awaiting approval for use in France; methylene blue-treated plasma is in routine use in Germany and Switzerland.

**Viral Inactivation of Blood Cell Concentrates**

Red Blood Cell Concentrates (RBCC) and Platelet Concentrates continue to harbor a small but defined risk of virus transmission, estimated for HIV, HBV, and HCV to be 1/225,000, 1/200,000, and 1/3,300 to 1/6,000, respectively, per unit infused. Additional improvements in donor selection and screening may reduce these risks somewhat. However, most patients are multiply transfused so that the risk per recipient typically will be 5- to 100-fold higher. Additionally, other viruses such as cytomegalovirus (CMV), human T-cell lymphotropic viruses (HTLV-I and HTLV-II) and parasites such as Yersinia enterocolitica and Trypanosoma cruzi may be present.

As compared with virus inactivation of blood protein products, sterilization of cellular products is more difficult because blood cells are more complex and fragile than proteins, and multiple viral forms are present, including cell-free virus, virus which is adherent to cell membranes, actively replicating virus, and latently infected cells. Nonetheless, since erythrocytes and platelets do not replicate, methods that modify membranes or nucleic acid may prove useful.

**Red Blood Cell Concentrates** - While numerous methods have been investigated, including use of β-propioloactone, nitrogen mustards, aryl diol epoxides, ozone, and halogenated oxidizing agents, the best results described thus far employ photodynamically active sensitizers and visible light. Although reaction mechanisms are complex, it seems likely that the major pathway leading to virus inactivation is the reaction of singlet oxygen and other reactive oxygen species with the envelop of viruses and/or viral nucleic acid. Early utilization of hematoporphyrin derivative has been improved upon by switching to photosensitizers which absorb light at a region of the spectrum where hemoglobin does not, i.e. above 630 nm. Treatment of RBCC with phthalocyanines, whose absorption maximum typically is between 660 and 690 nm, results in the inactivation of a variety of cell-free viruses, including vesicular stomatitis virus, Sendai virus, Sindbis virus, and HIV. Additionally, HIV which is actively replicating in host H9 cells and latently infected U1 cells are also inactivated. It seems likely that red cells are protected by their natural, anti-oxidative systems, including glutathione and catalase. Additional protection is afforded by exogenous addition of antioxidants. Red blood cells treated with aluminum phthalocyanine tetrasulfonate appear normal by electron microscopy and circulate
normally in a homologous baboon model. Recently, we have found that higher levels of
kill of cell-associated HIV and parasites could be obtained by substitution of aluminum
phthalocyanine tetrasulfonate with a silicon phthalocyanine (Pc 4). In vivo results with red
blood cells treated with this new phthalocyanine or with other photosensitizers are
pending.

**Platelet Concentrates** - Photodynamically active compounds such as those under
evaluation in the treatment of RBCC reduce platelet aggregation response to collagen and
to other agonists. In contrast, encouraging results have been obtained with psoralen
derivatives. Psoralens are naturally occurring furocoumarins found in many foods and
have been used therapeutically since antiquity. The principal reaction of psoralens on
exposure to long wavelength ultraviolet light is the cross-linking of nucleic acids.
Treatment of an oxygen depleted Platelet Concentrate with 8'-methoxypsoralen and UV
light was shown to inactivate $\geq 6.7 \log_{10}$ of E. coli, $\geq 6.9 \log_{10}$ of S. aureus, $\geq 7.3 \log_{10}$ PFU
of phage fd, 2.5 $\log_{10}$ of phage R17, and 5.1 $\log_{10}$ of feline leukemia virus. Platelet
morphology, process recovery, and response to the aggregation agent A23187 were
comparable to the untreated controls. The requirement to deoxygenate has been
overcome through the addition of quenchers of active oxygen species. Additionally, new
synthetic psoralens with increased reactivity with nucleic acids are being developed and
may serve to enhance reaction specificity further. In a thrombocytopenic rabbit model,
human platelets treated with aminomethyl-4,5,8-trimethylpsoralen (AMT) were equally
effective as the untreated control in shortening the bleeding time.

**Conclusion**

Blood and blood products have never been safer. However, the public's continuing
concern about blood viral safety and the differential safety profile between blood and other
pharmaceuticals demand that we continually improve. The achievements of the past are
laudable. Nonetheless, current risks fall well short of a standard of less than one
transmission per million units transfused, a realistic goal which I believe the transfusion
community should adopt. Pooled blood products which have been virally inactivated meet
this standard for most viruses, and use of a second viral elimination procedure which
complements the first will further ensure safety of these products. For single donor blood
products, improved screening systems may achieve this goal; however, screening systems
alone will never eliminate the so-called window period and screening tests cannot
anticipate new viruses or viral serotypes. Incorporation of viral inactivation procedures into
the manufacture of all blood products, including blood cell concentrates, overcomes the
weaknesses of screening procedures and the further development of virus inactivation
methodology should continue to be encouraged.

**Selected References**


