

Fig. 3. Human cytokine levels in recipient mice plasma. Plasma samples obtained from recipient mice injected with Mirasol-treated cells (T) or untreated cells (UT) were tested for the concentrations of the indicated cytokines. The data show the results when undiluted plasma was tested except for the levels of IFN- γ and IL-10, which were obtained with plasma diluted 1:10. Samples in which no cytokine was detected were recorded as 0.1 pg per mL.

TABLE 3. Amount and specificity of human immunoglobulin present in the plasma of recipient mice injected with untreated MNCs

Antibody isotype	Antibody specificity	Untreated cells	Treated cells
IgG	Not tested	5981 \pm 2781*	0.1 \pm 0.2*
IgM	Not tested	1390 \pm 845*	0.3 \pm 0.8*
IgG	Mouse RBCs	16.1 \pm 17.9†	0.3 \pm 0.4†
IgM	Mouse RBCs	30.8 \pm 21.7†	0.2 \pm 0.5†
IgG	Mouse WBCs	5.1 \pm 6.1†	0.5 \pm 0.3†
IgM	Mouse WBCs	8.9 \pm 7.3†	0.2 \pm 0.4†
IgG	Mouse PLTs	28.2 \pm 17.8†	0.3 \pm 0.6†
IgM	Mouse PLTs	25.6 \pm 23.5†	0.0 \pm 0.1†

* ng/mL.
† Percent positive cells.

mice was tested.⁴ These results indicated that Mirasol PRT-treated cells were unable to generate xenogeneic GVHD responses. Thus, it would be predicted that Mirasol PRT treatment would prevent the development of TAGVHD after the transfusion of treated blood products.

The standard current protocol for prevention of TAGVHD is to expose blood products to 25 Gy of gamma irradiation. Although this dose of gamma irradiation has been shown to be effective in preventing the proliferation of irradiated WBC, subsequent studies have indicated that 25 Gy of gamma irradiation is not sufficient to prevent the presentation of antigens by these cells.^{13,25} In contrast, Mirasol PRT treatment is able to completely inhibit all functions of WBCs including the ability to directly present antigen and induce proliferation of normal PBMCs in an MLC assay.¹¹ This would suggest that Mirasol PRT treat-

ment is able to prevent at least direct antigen presentation by the treated cells, indicating that Mirasol PRT treatment could potentially prevent the induction of alloantibodies after the transfusion of treated blood products. This would distinguish this treatment from gamma irradiation, which does not interfere with alloimmunization and the induction of alloantibodies.^{13,25}

These studies also provide additional information about the xenogeneic GVHD response in Rag2^{-/-} double-knockout recipient mice. A previous study had shown that injection of human PBMCs into Rag2^{-/-} double-knockout recipient mice resulted in enhanced peripheral reconstitution with human T cells.⁴ Separation of donor PBMCs into different subpopulations showed that both CD4+ and CD8+ cells were required to induce lethal GVHD in this model.⁴ Although it took longer to develop xenogeneic GVHD than had

been published, it was confirmed that it was primarily human T cells including both CD4+ and CD8+ cells that reconstituted the peripheral lymphoid compartments. One explanation for the delayed kinetics may be due to intraperitoneal injection instead of intravenous injection of the donor cells. Another possibility is that we did not bleed our recipient mice weekly, which could induce leukopenia and facilitate reconstitution with the human donor cells. Human CD19+ cells were the primary cells found in the liver and in any intestinal lymphoid tissue that was observed. The preferential location of B cells in the peritoneal cavity could represent an inability of the B cells to home to other sites in the murine recipient after intraperitoneal injection. Another possibility is that this tissue could represent the beginnings of an expansion of Epstein-Barr virus-seropositive B cells that has been previously observed in studies of immunodeficient SCID mice.^{6,26} These cells represent an uncontrolled expansion of these cells as occurs in posttransplant lymphoproliferative disorder.²⁷

Characterization of human cytokines present in the serum at early time points after injection of the human cells into Rag2^{-/-} double-knockout recipients found high levels of IL-1 β , IL-2, IL-15, and IL-18; intermediate levels of IL-6 and IL-10; and very low levels of IL-4, IL-8, IFN- γ , and TNF- α .⁴ The lack of human cytokines in the plasma of recipients of cells treated with Mirasol PRT was consistent with the lack of human cell chimerism observed with other approaches. Measurement of the

level of cytokines in the plasma of recipients with end-stage GVHD contained a very different mixture of cytokines because very high levels of IFN- γ , high levels of IL-10, and low levels of IL-5, TNF- α , IL-1 β , IL-6, IL-8, IL-12p70 were detected. Concordant expression of high levels of both IL-10 and IFN- γ have been observed in autologous GVHD in humans.²⁸ Autologous GVHD is induced by a short course of cyclosporine in recipients of autologous transplants. Autologous GVHD is the result of cytolytic CD8+ cell responses whose T-cell receptors recognize MHC class II molecules containing the CLIP peptide. Polymorphisms in the IL-10 promoter were also found to correlate with the ability to generate autologous GVHD responses in patients.²⁸ Thus the xenogeneic GVHD responses in these recipients may be useful for modeling autologous GVHD responses.

The results indicate that both human CD4+ and CD8+ T cells are found in the recipient mice injected with untreated cells and that a large percentage of these cells express CD107a, indicative of CD4+ and CD8+ cells that had previously carried out granule exocytosis. There is evidence indicating that CD4+ cells can act as cytolytic cells in murine GVHD responses.²⁹ In these studies, the cytolytic CD4+ cells appeared to lyse peripheral blood progenitor cells in the marrow. This would provide one explanation for the decrease in Hct levels observed in the recipients of untreated MNCs. Although the antigens recognized by human T cells present in the Rag2^{-/-} γ c^{-/-} double-knockout recipient mice undergoing GVHD have not been determined, previous studies have suggested that human T cells recognize xenogeneic H-2 antigens expressed by murine antigen-presenting cells.^{30,31}

Another possible explanation for the decreased Hct levels in the recipients of untreated MNCs could be the production of xenoreactive antibodies by the human cells. Published studies had indicated that the injection of human cells results in production of xenoreactive antibodies that bind to multiple antigens expressed by murine RBC.³² We also observed that there were high levels of human immunoglobulins in the serum of the recipients, and that these immunoglobulins contained primarily IgM antibodies with specificity toward murine RBCs and PLTs but not WBCs. The activation of complement by the IgM bound to the RBCs could facilitate the elimination of the murine RBCs.

In summary, these experiments demonstrate that the injection of untreated MNCs into Rag2^{-/-} γ c^{-/-} double-knockout recipient mice induces a xenogeneic GVHD response that resembles the responses seen in TAGVHD. Mirasol PRT-treated donor cells are unable to generate xenogeneic GVHD when injected into Rag2^{-/-} γ c^{-/-} double-knockout recipient mice. This finding is consistent the previous finding that the Mirasol PRT-treated cells are functionally inactive in *in vitro* assays.¹¹ These findings suggest that a single treatment would be able not only to

inactivate pathogens present in blood products but also prevent the immunologic consequences such as TAGVHD that can occur after blood transfusion.

REFERENCES

1. Vamvakas EC, Blajchman MA. Deleterious clinical effects of transfusion-associated immunomodulation: fact or fiction? *Blood* 2001;97:1180-95.
2. Lee JH, Klein H. From leukocyte reduction to leukocyte transfusion: the immunological effects of transfused leukocytes. *Bailliere's Clin Hematol* 2000;13:585-600.
3. Gould DS, Auchincloss HJ. Direct and indirect recognition: the role of MHC antigens in graft rejection. *Immunol Today* 1999;20:77-82.
4. van Rijn R, Simonetti E, Hagenbeek A, et al. A new xenograft model for graft-versus-host disease by intravenous transfer of human peripheral blood mononuclear cells in RAG2^{-/-} γ c^{-/-} double mutant mice. *Blood* 2003;102:2522-31.
5. Fast L, Semple J, DiLeone G, et al. Inhibition of xenogeneic graft-versus-host disease by PEN110 treatment of donor human peripheral blood mononuclear cells. *Transfusion* 2004;44:282-5.
6. Mosier D, Guilizia R, Baird S, Wilson D. Transfer of a functional human immune system to mice with severe combined immunodeficiency. *Nature* 1988;335:256-9.
7. Barry T, Jones D, Richter C, Haynes B. Successful engraftment of human postnatal thymus in severe combined immune deficient (SCID) mice: differential engraftment of thymic components with irradiation versus anti-asialo GM1 immunosuppressive regimens. *J Exp Med* 1991;173:167-80.
8. Sandhu J, Gorczyński R, Shpitz B, et al. A human model of xenogeneic graft-versus-host disease in SCID mice engrafted with human peripheral blood lymphocytes. *Transplantation* 1995;60:179-84.
9. Fraser C, Chen B, Webb S, et al. Circulation of human hematopoietic cells in severe combined immunodeficient mice after Cl2MDP-liposome-mediated macrophage depletion. *Blood* 1995;86:183-92.
10. Kumar V, Lockerbie O, Ruane P, et al. Riboflavin and UV-light based pathogen reduction: extent and consequences of DNA damage at the molecular. *Photochem Photobiol* 2004;80:15-21.
11. Fast L, DiLeone GJ, Li J, Goodrich R. Functional inactivation of white blood cells by Mirasol treatment. *Transfusion* 2006;46:642-8.
12. Fast L, DiLeone G, Edson C, Purmal A. PEN110 treatment functionally inactivates the PBMNCs present in RBC units: comparison to the effects of exposure to gamma irradiation. *Transfusion* 2002;42:1318-25.
13. Fast L. The effect of exposing murine splenocytes to UVB light, psoralen plus UVA light or gamma-irradiation on *in vitro* and *in vivo* immune responses. *Transfusion* 2003;43:576-83.

14. Alter G, Malenfant J, Altfeld M. CD107a as a functional marker for the identification of natural killer cell activity. *J Immunol Methods* 2004;294:15-22.
15. Betts M, Brenchley J, Price D, et al. Sensitive and viable identification of antigen-specific CD8+ T cells by a flow cytometric assay for degranulation. *J Immunol Methods* 2003;281:65-78.
16. Grossman W, Verbsky J, Tollefsen B, et al. Differential expression of granzymes A and B in human cytotoxic lymphocyte subsets and T regulatory cells. *Blood* 2004;104:2840-8.
17. Grossman W, Verbsky J, Barchet W, et al. Human T regulatory cells can use the perforin pathway to cause autologous target cell death. *Immunity* 2004;21:589-601.
18. AuBuchon JJ, Herschel L, Roger J, et al. Efficacy of apheresis platelets treated with riboflavin and ultraviolet light for pathogen reduction. *Transfusion* 2005;45:1335-41.
19. Ruane P, Edrich R, Gampp D, et al. Photochemical inactivation of selected viruses and bacteria in platelet concentrates using riboflavin and light. *Transfusion* 2004;44:877-85.
20. Kumar V, Motheral T, Luzniak G, Goodrich R. Mirasol pathogen reduction technology: riboflavin-based plasma process inactivates intracellular and extracellular human immunodeficiency. *J Clin Apher* 2003;18:84.
21. Goodrich L, Douglas I, Urisote M. Riboflavin photoinactivation procedure inactivates significant levels of bacteria and produces a culture negative product. *Transfusion* 2002;42:16S.
22. Goodrich LM, Ghielli M, Hansen E, et al. Riboflavin and UV light inactivate enveloped and non-enveloped viruses in platelet concentrates which maintain cell quality. *Vox Sang* 2002;83:111.
23. Goodrich R. Inactivation of viruses in blood products (platelets, plasma, and red cells). *Transfus Clin Biol* 2001;8:103S.
24. Andre-Schmutz I, Deist FL, Hacein-Bey-Abina S, et al. Immune reconstitution without graft-versus-host disease after haemopoietic stem-cell transplantation: a phase 1/2 study. *Lancet* 2002;360:130-7.
25. Ohto H. Gamma radiation does not prevent transfusion-induced HLA alloimmunization. *Transfusion* 1997;37:878-9.
26. Kamel-Reid S, Dick J. Engraftment of immune-deficient mice with human hematopoietic stem cells. *Science* 1988;242:1706-9.
27. Mamzer-Bruneel MF, Bourquelot P, Hermine O, Legendre C, Kreis H. Treatment and prognosis of post-transplant lymphoproliferative disease. *Ann Transplant* 1997;2:42-8.
28. Miura Y, Thoburn CJ, Bright EC, et al. Cytokine and chemokine profiles in autologous graft-versus-host disease (GVHD): interleukin 10 and interferon γ may be critical mediators for the development of autologous GVHD. *Blood* 2002;100:2650-8.
29. Sprent J, Suth CD, Agus D, et al. Profound atrophy of the bone marrow reflecting major histocompatibility complex class II-restricted destruction of stem cells by CD4+ cells. *J Exp Med* 1994;180:307-17.
30. Lindahl K, Bach F. Human lymphocytes recognise mouse alloantigens. *Nature* 1975;254:607-9.
31. Lindahl K, Bach F. Genetic and cellular aspects of xenogeneic mixed leukocyte culture reaction. *J Exp Med* 1976;144:305-18.
32. Williams SS, Umemoto T, Kida H, Repasky EA, Bankert RB. Engraftment of human peripheral blood leukocytes into severe combined immunodeficient mice results in the long term and dynamic production of human xenoreactive antibodies. *J Immunol* 1992;149:2830-6. ■

TRANSFUSION MEDICINE REVIEWS

Vol 22, No 2

April 2008

Pathogen Reduction: A Precautionary Principle Paradigm

Harvey J. Alter

Although remarkable advances have been made in the prevention of the major transfusion-transmitted diseases, long intervals have transpired between the first recognition of transfusion risk and the implementation of a preventive strategy. For hepatitis B virus, that interval was 30 years; for non-A, non-B/hepatitis C virus, 15 years; and for human immunodeficiency virus, West Nile virus, *Trypanosoma cruzi*, and bacteria, 3, 4, 5, and 18 years, respectively. In our existing reactive approach, there is a fundamental and inevitable delay before we can react; and thus, infections are destined to occur. The continued emergence or reemergence of transfusion-transmitted infections calls for a new paradigm of preemptive pathogen reduction (PR). Two PR systems, psoralen/UV-A and riboflavin/

UV-A, have shown efficacy and safety for platelets and plasma; and psoralen/UV-A technology has been successfully implemented for platelets in Europe. Pathogen reduction can eliminate or reduce the risk for any nucleic acid containing agent, including bacteria, and thus will be effective for all but prion diseases. It is possible to introduce PR for platelets and plasma now and to concentrate resources on developing PR for red cells. This will require an intellectual and financial commitment from the National Institutes of Health, the Food and Drug Administration, industry, and the blood bank establishment, just as occurred for nucleic acid testing (NAT) technology. This can be done if there is sufficient will to do it.

© 2008 Published by Elsevier Inc.

IN THE WAKE of the human immunodeficiency virus (HIV) tragedy, the Food and Drug Administration (FDA) and US blood establishments have endorsed the *precautionary principle* that states that "for situations of scientific uncertainty, the possibility of risk should be taken into account in the absence of proof to the contrary." As a corollary, the precautionary principle asserts that "measures need to be taken to face potential serious risks." Yet pathogen reduction (PR), perhaps the quintessential example of the precautionary principle, has not been embraced. Pathogen reduction calls for a new paradigm in transfusion safety, namely, the transition from a reactive to a proactive and preemptive strategy for the prevention of transfusion-transmitted diseases. Pathogen reduction may initially add cost and complexity to blood processing, but ultimately will not only offer maximum safety, but also will prove to be cost-neutral and possibly cost-saving.

The decline in transfusion-associated hepatitis incidence from 30% to near zero over the course of 3 decades has been considered one of the major

triumphs in transfusion medicine.¹ However, viewed in the present context, it can also be seen as one of the major failures of transfusion medicine because no preemptive viral reduction strategy was in place and because decades passed before the extent of the hepatitis risk was defined, the causative agents discovered, and proper testing strategies implemented. A vast number of cases occurred before we could, or chose to, do anything about it. Based on prospectively determined

From the Department of Transfusion Medicine, Warren Grant Magnuson Clinical Center, National Institutes of Health, Bethesda, MD.

Address reprint requests to Harvey J. Alter, MD, FACP, Chief, Infectious Disease Section, Department of Transfusion Medicine, Bldg 10, Room 1C-711, NIH, 10 Center Drive, Bethesda, MD 20892.

E-mail: halter@dtm.cc.nih.gov

0887-7963/08/\$ - see front matter

© 2008 Published by Elsevier Inc.

doi: 10.1016/j.tmr.2008.01.001

Table 1. Time Interval Between Recognition of Risk and Implementation of a Donor Screening Assay

Agent	Recognized as a transfusion risk	First screening assay	Interval (y)
HBV	1940	1970	30
NANB/HCV	1975	1990	15
HIV	1982	1985	3
WNV	2002 (1999)*	2003	1 (4)*
Chagas	2002	2007	5
Bacteria	1986	2004	18

Abbreviations: HBV, hepatitis B virus; NANB, non-A, non-B agent; HIV, human immunodeficiency virus; WNV, West Nile virus; Chagas, *T. cruzi* infection.

* Suspected, but not proven, in 1999.

hepatitis incidence rates¹ and the number of blood transfusions nationwide in the United States, it can be estimated that there were 4.8 million transfusion-transmitted hepatitis C virus (HCV) infections between 1970 and 1990; and based on a 20% incidence of severe outcomes, these might have resulted in 768 000 cases of cirrhosis. Clearly, not all these cases of cirrhosis occurred because many transfusion recipients died of underlying diseases before the severe chronic manifestations of hepatitis could ensue. This does not diminish the fact that allogeneic transfusions placed these recipients at potential risk for a fatal disease. These projections are sobering, even if they are off by a factor of 10.

Historically, there has been a very long interval between the first recognition that a disease is transfusion-transmitted and the eventual implementation of a donor-screening test to prevent that transmission (Table 1). For the hepatitis B virus (HBV), the interval between recognition of transfusion-transmission and implementation of the first assays for HBV, the Australia antigen, was approximately 30 years; and for non-A, non-B/HCV hepatitis, it was 15 years. For HIV, the interval was reduced to 3 years; however, in that comparatively brief interval, more than 14 000 transfusion-transmitted, predominantly fatal HIV infections occurred in the United States alone.² We generally consider that the interval from recognition to implementation for West Nile virus (WNV) was from 2002 to 2003; however, retrospective analysis of the 1999 WNV epidemic in New York City made it highly probable that WNV could be transmitted by transfusion,³ so that the true implementation interval was approximately 4 years. In addition, testing donations for *Trypanosoma cruzi* (Chagas disease) was recommended by the FDA's Blood

Products Advisory Committee in 2002; and yet the first reliable test was not introduced until 2007. Furthermore, the relatively frequent transmission of bacteria by room temperature-stored platelets was evident by 1986; but it was not until 18 years later (2004) that routine bacteriologic testing of platelets was introduced in the United States. The inherent problem is that in a reactive strategy to pathogen risks, there is a fundamental and inevitable delay between the recognition of risk and the prevention of that risk. Thus, transfusion-transmitted infections are destined to occur before we can adequately react. Perhaps William Murphy phrased it best when he stated, "this effect, that a new or poorly understood disease with a long preclinical phase can be spread among humans by transfusion for many years, and perhaps extensively, before the consequences become apparent is a fixed and inevitable property of transfusion medicine."⁴

Nonetheless, on a more positive note, based on prospectively determined hepatitis incidence figures,¹ the introduction of anti-HCV screening in 1990 is projected to have prevented 1.2 million cases of transfusion-associated hepatitis and 192 000 potential cases of cirrhosis in the decade of the 1990s. Furthermore, increasingly sensitive serologic and nucleic acid tests for HCV and HIV have reduced these risks dramatically. In the United States, the risks for the 3 major transfusion-transmitted viruses in 2006 were 1 in 1.6 million for HCV, 1 in 1.8 million for HIV, and 1 in 269 000 for HBV, as calculated by the incidence rate-window period model and approximately the same using the minipool nucleic acid testing (NAT) yield model.⁵ In Canada, O'Brien et al.,⁶ using the incidence rate-window period model, reported a residual risk of 1 in 7.8 million for HCV, 1 in 2.3 million for HIV, and 1 in 153 000 for HBV. Although HBV transmissions occur much more frequently than HCV or HIV, this estimate is based on antibody to hepatitis B core antigen (anti-HBc) seroconversion and not on the development of the hepatitis B surface antigen (HBsAg) carrier state or clinical disease. Actual cases of transfusion-associated hepatitis B are quite rare.

Currently, the major infectious risks are zoonotic in origin, wherein animal viruses have been introduced to humans as an incidental host, either through the food chain, as in variant Creutzfeldt-Jakob disease, or through vector transmission. Mosquitoes, ticks, and other insect vectors are

injecting diseases into us at alarming frequency; and most, if not all, of these are then secondarily transmitted by transfusion. These vector-borne and transfusion-borne diseases include malaria, dengue fever, WNV-associated fever and meningoencephalitis, babesiosis, ehrlichiosis, Colorado tick fever, and Chagas disease. Conclusive evidence is missing; but Lyme disease, visceral leishmaniasis, and flavivirus-induced encephalitis might also prove to be transfusion-transmitted.

The history of transfusion-transmitted WNV infection is a case in point. Before the implementation of WNV testing in 2003, there were 23 documented transfusion-transmitted clinical cases reported in the United States; and based on the Centers for Disease Control and Prevention conversion factor of 140 asymptomatic cases to each clinical case,⁷ it can be estimated that there were an additional 3200 subclinical transfusion-transmitted cases in 2002. It is commendable that WNV NAT testing began under IND only 8 months after the first documented transfusion-transmission. However, in retrospect, it was projected from the New York City epidemic in 1999 that transfusion-transmitted WNV cases were likely.³ However, because the evidence was indirect and WNV epidemics were known to be intermittent in nature, no definitive action was taken at that time. Overall, since 2002, there have been 32 documented transfusion-associated cases of clinical WNV infection and 4480 projected transmissions in the United States. Remarkably, none of these cases would have occurred had a PR system been in place at the time, as evidenced by the absence of cases associated with the transfusion of solvent-detergent-treated plasma. Once implemented in 2003, WNV testing of donor blood has been highly effective. In the American Red Cross experience, WNV NAT prevented the release and transfusion of at least 1000 potentially infectious components from 519 confirmed positive blood donors in 2003 and 2004.⁸ There have been only 9 WNV transmissions since testing was implemented, each representing a window period donation that failed to be detected in the minipool testing format being used.

There is current concern that the WNV story may be replayed by the dengue fever virus (DFV) because the agents and predisposing elements are similar. Globally, DFV causes 100 million cases of dengue fever, 250 000 cases of dengue hemorrhagic

fever, and 25 000 deaths annually. It is transmitted by the *Aedes* mosquito and is found in more than 100 countries with a rapidly expanding distribution that could easily follow the path of WNV to North America. The DFV has a median viremia of 5 days, and most cases are asymptomatic; RNA levels range from 10^5 to 10^9 copies per milliliter. Thus, dengue has all the characteristics of a transfusion-transmitted agent; and its vector is already present in North America. Thus far, there have been 2 transfusion-transmitted cases, 2 transplant-related cases, and 7 nosocomial dengue transmissions that have been documented.⁹ Undoubtedly, many more transfusion-associated dengue cases occur in endemic areas; but these are masked by the high background infection rate. Fortunately, the epidemiologic pattern of this agent differs from WNV in that it does not have an intermediary bird host that would facilitate its spread.

An agent of particular current interest is human herpesvirus (HHV)-8, important not only for its potential to transmit Kaposi sarcoma (KS) but also because it is critical to decisions regarding the donor reentry of men who have sex with men (MSM). The prevalence of HHV-8 antibody is 2% to 4% in blood donors,¹⁰ 2% to 10% in the general population, 12% to 16% in HIV-negative MSMs, 40% to 50% in HIV-positive MSMs, and >95% in patients with KS (Dollard SC, personal communication). There have been several case reports and epidemiologic insights to suggest that HHV-8 can be transfusion-transmitted, but the best data indicating the transfusion-transmission of HHV-8 are in the study by Hladik and associates at the Centers for Disease Control and Prevention¹¹ who followed 1811 transfusion recipients in an HHV-8 endemic area of Uganda. The risk of seroconversion was significantly higher among recipients of seropositive blood (excess risk, 2.7%; $P < .005$); and the increase was seen primarily in those who seroconverted for anti-HHV-8 between 3 and 10 weeks posttransfusion, an interval highly suggestive of transfusion-transmission.

In the final analysis, any agent that even transiently traverses the human circulation during an asymptomatic phase of infection is a threat to be transfusion-transmitted. The likelihood of that transmission is highly dependent on the duration of viremia ("agentemia"), and the level of concern is dependent upon the severity of the ensuing disease. Agents for which there are no routine screening

measures in place and that pose a documented or potential transfusion risk in this model include malaria sp, dengue and other arboviruses, HHV-8 (KS virus), cytomegalovirus (CMV) and other herpes viruses, parvovirus B-19 (erythrovirus), human papilloma virus, Colorado tick fever virus, *Babesia microti* (*Babesia*), *Ehrlichia* and other *Rickettsia*, *Borrelia burgdorferi* (Lyme disease), chikungunya virus, hepatitis A virus, and variant Creutzfeldt-Jakob disease. A comprehensive analysis of 68 potential/known transfusion-transmitted agents is being prepared for publication by a task force of the American Association of Blood Banks (AABB) and should be accessible sometime during 2008 (Stramer S, personal communication).

This vast array of potential microbiological threats requires continuous surveillance, clinical assessment of the magnitude of each identified risk, and then, where possible, testing or other strategies to limit risk of the most clinically significant agents. This is thus an agent by agent process that is inefficient, insensitive, often controversial in its decisions, and inevitably applied only after clinical disease has occurred. It is a reactive strategy that requires demonstrable risk before preventive measures can be implemented. A more encompassing, efficient, and intuitively appealing option is a preemptive approach that includes PR. Almost all of the aforementioned agents and many others can be reduced to nonpathogenic levels by nucleic acid intercalating agents such as the psoralens¹² and riboflavin¹³ in the presence of UV-A light. Shown in Table 2 are known log reductions for psoralen/UV-A, and much the same could be shown for riboflavin/UV-A.

Pathogen reduction has multiple proven advantages in that it: (1) effectively inactivates most clinically relevant viruses whether RNA or DNA, single-stranded or double-stranded, enveloped or nonenveloped, and intracellular or extracellular; (2) inactivates clinically relevant gram-positive and

gram-negative bacteria; (3) inactivates all the spirochetes, *Rickettsia*, and protozoa of known transfusion relevance; (4) inactivates lymphocytes and thus prevents transfusion-associated graft-versus-host disease (GVHD); and (5) offers probable preemptive protection against pathogenic, potentially lethal agents that will inevitably emerge in the future.

There are also impediments to the implementation of PR that to this point have limited its widespread application, as follows: (1) decreased product yield for platelets in the range of 10% to 15%; (2) insufficient kill of some high-titer, nonenveloped agents such as hepatitis A virus (HAV) and parvovirus B-19. However, antibodies to these agents are common in the recipient population, and documented transmissions are exceedingly rare; (3) concern over potential toxicity, although none are known for riboflavin and toxicity of the psoralens is theoretical at the low residual doses transfused. For both agents, there appears to be a wide safety margin; (4) most significantly, there is at present no single PR system that can be applied to all blood products and, particularly, no proven system for PR of whole blood and packed red cells; and (5) anticipated high cost.

Although there are no methods for the PR of red blood cells (RBCs) that have been proven effective and safe in appropriate clinical trials, if the evolving technologies establish their efficacy and multicomponent PR can be implemented, then there are many potential savings that would offset the cost of the process itself, including (1) reducing or eliminating the future need for additional donor screening assays such as testing for HHV-8, *Babesia*, *Ehrlichia*, dengue, and malaria; (2) eliminating some current assays including anti-HBc, WNV, *T cruzi* (Chagas), and syphilis; (3) eliminating the bacterial testing of platelets; (4) discontinuing irradiation of blood products; (5) discontinuing leukoreduction; (6) allowing for the continuation of

Table 2. Inactivation of Infectious Agents in Plasma and Platelets Using Psoralen/UV-A

Classification	Agents	Log reduction
Virus (enveloped)	HIV-1/2, HTLV-I/II, HBV, DHBV, HCV, BVDV, WNV, CMV, SARS-CoV, vaccinia	>4.5 to >6.8
Virus (nonenveloped)	Human adenovirus-5, bluetongue, parvovirus B-19, HAV	>5.1 to >6.8 3.5 to >5.0
Bacteria	Gram + and gram-	>7.3
Spirochetes	<i>Treponema pallidum</i> , <i>B burgdorferi</i>	>5.9 to >10.6
Protozoa	<i>Plasmodium falciparum</i> , <i>T cruzi</i> , <i>B microti</i>	>5.0 to >6.9

Abbreviations: HTLV, human T lymphotropic virus; BVDV, bovine diarrheal virus.

minipool testing rather than the probable evolution to individual donor testing; and (7) reducing donor exclusions based on geography (malaria). Cumulatively, these measures could result in vast savings that should offset the implementation costs of PR. However, these savings can only be realized after procedures for the PR of RBC products are fully operational.

Perhaps the key immediate issue is not the efficacy of PR, where the evidence is substantial, and not even its safety, where toxicity remains theoretical, but rather whether we should introduce PR reduction for platelets and single-donor plasma before a system is in place to inactivate pathogens in RBC products. This is a difficult conundrum, and opinion on this issue is sharply divided. However, it is known that many patients receive repeated, often-daily platelet transfusions that are only intermittently accompanied by RBC transfusions and also that many centers pool platelet products, vastly increasing the recipient exposure risk.

It is also probable that if we wait for the complete PR package, another 5 to 10 years will elapse before licensure and implementation; and in that time, innumerable platelet and plasma infusions will continue to transmit infections to recipients that could have easily been prevented. If such transfusion-transmission infections are accompanied by significant disease, then further transfusion-transmitted tragedies could ensue. I believe that the precautionary principle and the moral imperative dictate that we implement what we have, even if they are less than perfect. Admittedly, there is the other side of the coin in that platelet and single-donor plasma inactivation, in the absence of RBC inactivation, will not reap the financial offsets of a complete PR system and in that RBC products will continue to transmit disease. Furthermore, it is known that transfusion-transmitted pathogens are only one part of the risk equation and, now, not even the largest part. However, I would counter that preventing human error, controlling transfusion-related acute lung injury, and PR are not mutually exclusive and all ways of reducing transfusion risk should be pursued with equal vigor.

The solvent-detergent treatment of plasma and its derivatives has established the principle that PR of even a single blood component is highly beneficial and has established the enormous value of a preemptive PR strategy. Universal inactivation of plasma derivatives has rendered the formerly

highest-risk blood products now to be the safest. As blood transfusion services scrambled to meet the threat of WNV, how reassured plasma manufacturers were to know that they had this agent preemptively covered, as they would for DFV or any other lipid-encapsulated agent that threatens the blood supply. Had solvent detergent treatment of plasma derivatives been implemented in the early 1980s, most HIV and HCV cases that devastated the hemophiliac population could have been avoided. This statement is being made not to cast retrospective blame, but to take a lesson from history and to illustrate the value of having a protective preemptive mechanism in place before the next agent strikes.

There is great potential risk in delaying implementation of PR while waiting for absolute evidence and a perfect system to be put into place. Waiting is a calculated risk that defies the precautionary principle. Can we chance the possibility that a new lethal agent will enter the blood supply and replay the HIV tragedy? Can we face future generations and say we did all that we could do at the time? I believe the time has come to act. At present, there are 2 technologies, psoralen/UV-A and riboflavin/UV-A, that would bring the same level of safety to platelets as currently exists for commercial plasma and its derivatives. Evidence for the efficacy and safety of PR for platelets and single-donor plasma products is sufficient,^{14,15} if not overwhelming; and the European experience with psoralen/UV-A-treated platelets has proven the practicality and safety of this approach to transfusion-transmitted disease prevention.¹⁶ Whether or not one concurs with the need to rapidly introduce PR for platelets and nonfractionated plasma in the United States and Canada, the more important and, I believe, crucial message is that we have to establish a mind-set that says PR of *all* blood products is a laudatory and achievable goal and that we need to invest ourselves emotionally, intellectually, and financially to make this happen. The blood bank establishment, the National Institutes of Health, the FDA, and industry have to make this concept a priority and then work in concert to devote substantial resources and energy to achieve this goal, just as they did for viral nucleic acid testing. Only then will it happen!

I was in that packed hotel ballroom in 1994 when Dr David Kessler urged blood banks to develop NAT for routine donor screening. His talk raised

eyebrows and great skepticism; but because of his position of authority, it drove the system, generated government-industry collaboration, and resulted in the remarkably rapid development of practical NAT assays that have been an enormous addition to

blood safety. I am no David Kessler; but I admonish and encourage you to say this is the right thing to do and that we have to find a way to do it. We have to bite the bullet. Fortunately, in this case, it is a magic bullet.

REFERENCES

1. Alter HJ, Houghton M: Hepatitis C virus and eliminating post-transfusion hepatitis. *Nat Med* 6:12-16, 2000
2. Selik RM, Ward JW, Buehler JW: Trends in transfusion-associated acquired immune deficiency syndrome in the United States, 1982 through 1991. *Transfusion* 33:890-893, 1993
3. Biggerstaff BJ, Peterson LR: Estimated risk of West Nile virus transmission through blood transfusion during an epidemic in Queens, New York City. *Transfusion* 42:1019-1026, 2002
4. Murphy W: Managing threats rather than risks in blood transfusion: Robust design for a complex system. *Transfusion* 46:2011-2013, 2006
5. Busch MP: Transfusion transmitted viral infections: Building bridges to transfusion medicine to reduce risks and understand epidemiology and pathogenesis. *Transfusion* 46:1624-1640, 2006
6. O'Brien SF, Yi Q-L, Fan W, et al: Current incidence and estimated residual risk of transfusion-transmitted infections in donations made to Canadian Blood Services. *Transfusion* 47:316-325, 2007
7. Centers for Disease Control and Prevention (CDC) serosurveys for West Nile virus infection—New York and Connecticut counties 2000. *MMWR Morb Mortal Wkly Rep* 50:37-39, 2001
8. Stramer SL, Fang CT, Foster GA, et al: West Nile virus among blood donors in the United States, 2003 and 2004. *N Engl J Med* 353:451-459, 2005
9. Centers for Disease Control and Prevention. Dengue Fever. www.cdc.gov/ncidod/dvbid/dengue. 12-19-2006.
10. Pellett PE, Wright DJ, Engels EA, et al: Multicenter comparison of serologic assays and estimation of human herpesvirus 8 seroprevalence among US blood donors. *Transfusion* 43:1260-1268, 2003
11. Hladik W, Dollard SC, Mermin J: Transmission of human herpesvirus 8 by blood transfusion. *N Engl J Med* 355:1331-1338, 2006
12. Allain JP, Bianco C, Blajchman MA, et al: Protecting the blood supply from emerging pathogens: The role of pathogen inactivation. *Transfus Med Rev* 19:110-126, 2005
13. Goodrich RP: The use of riboflavin for the inactivation of pathogens in blood products. *Vox Sang* 78(Suppl 2):211-215, 2000
14. Slichter SJ, Raife TJ, Davis K, et al: Platelets photochemically treated with amatosalen HCl and ultraviolet A light correct prolonged bleeding times in patients with thrombocytopenia. *Transfusion* 46:731-740, 2006
15. Mintz PD, Bass NM, Petz LD, et al: Photochemically treated fresh frozen plasma for transfusion of patients with acquired coagulopathy of liver disease. *Blood* 107:3753-3760, 2006
16. van Rhenen D, Gulliksson H, Cazenave JP, et al: Transfusion of pooled buffy coat platelet components prepared with photochemical pathogen inactivation treatment: The euroSPRITE trial. *Blood* 101:2426-2433, 2003

バイオワン株式会社回答

4月8日運営委員会・安全技術調査会合同委員会後

追加質問事項2(各社共通)

インターセプトブラッドシステム(S-59) バイオワン株式会社

1. EU主要国等、各社の不活化技術を導入しているそれぞれの国における供給の実態。輸血用血液製剤の全供給数(不活化技術を適用している製剤と適用していない製剤の合計)、そのうち不活化技術を適用している製剤の割合は何%か。また、供給はどのように行われているか(供給先、医師の希望により供給できるのか等)。

国	血小板供給数		血漿供給数	
	全数	不活化率	全数	不活化率
ベルギー	70,000	100% ^{*1}	110,000	
フランス	230,000	約10%[2007年]	245,000	100% ^{*2}
ドイツ	400,000		1,200,000	
イタリア	120,000		605,000	
スペイン	215,000		275,000	
スウェーデン	35,000		120,000	
ノルウェイ	20,000		30,000	
ロシア	75,000		260,000	

- * 1: ベルギーは血小板の 100%不活化導入が決定されているが、未政府状態が続いており、大臣就任後 100%実施になる。保険適用についても実施が遅れている。血漿についてはすでに2センターで IBS の日常的使用を開始。
- * 2: フランスは規制により血漿の 100%不活化を要求されている。IBSによる不活化率は未確認(アルサスなど3か所で MB から IBS に変更)
- * 3. ドイツではリューベック、フランクフルトで血小板用 IBS の日常的使用開始。
- * 4. イタリア、スペインでは地域により採用が始まっている。採用の判断は血液センターによる。昨年北イタリアで Chikungunya が発生した際には既に IBS を採用していたため血小板の供給に支障は生じなかった。
- * 5. ノルウェー、スウェーデンでは血液センターにより採用の判断をしている。不活化の費用は全体治療に必要な保険の範囲内で負担可能。
- * 6. ロシアは医師、患者の判断により不活化した製剤を血液供給者に要求。

供給方法

1) ベルギー

その地域の血液センターが提供する血液製剤をそのまま使用。血液センターによって供給する製剤が不活化されているかいないか異なっているが2008年中に100%不活化した血小板が供給される予定。不活化は100%IBSによる。

2) フランス

採用する血液センターは100%採用になっているため地域によって不活化されているかされていないか決まる。

3) ドイツ

血液製剤はドイツ赤十字(75-80%)、病院/大学(10-15%)、供給民間会社(10%以下)から供給されている。血小板は原則 Buffy Coat 血小板で成分採血血小板は理由をつけて医師の要求がある場合に限られる。ガンマー線照射も骨髄移植患者など特に必要な患者に限って実施している。現在は成分採血の際に IBS を採用しているが(リユーベック)今後は Buffy Coat にも IBS の採用を広げている予定です。フランクフルト血液センターで Hemovigilance の体制を整えて実施予定。

2. 各社の不活化技術を適用する前の製剤に白血球除去を行った場合と、不活化技術を適用した製剤に白血球除去を行わなかった場合を比較して、GVHD等の副作用の推移はどのようになっているか。

現在 白血球除去を行わずに不活化処理を実施している国はない。

白血球除去導入実態

ベルギー、フランス、ドイツ、スウェーデン、スペイン、ノルウェイ 100%

イタリア 約70%

ロシア 約20%

白除と、白除＋不活化の副作用の比較は下記情報がある。

InterCept による不活化導入前後の副作用の比較(Dr. Osselaer ベルギー)

血液製剤	対照期間(不活化処理前)		INTERCEPT 不活化処理期間	
	血小板(未処理)	赤血球(未処理)	血小板(不活化)	赤血球(未処理)
調査期間	18ヶ月	18ヶ月	18ヶ月	18ヶ月
輸血数	3529	9551	4051	11493
副作用	1.3%	0.4%	0.9%	0.4%

血小板では、不活化処理前の期間は細菌検査、γ線照射、CMV検査を実施していたが、不活化処理導入後これらの検査、処理は実施していない。

赤血球は不活化処理を行っていないので、両期間とも副作用の発生率に変化はない。

一方、血小板は不活化処理導入後、上記検査、照射を廃止したにもかかわらず、副作用の発生率が減少し統計的に有意差が認められている(p 0.002)

Alsace(フランス)

Adverse transfusion reactions during platelet concentrates (PC) transfused at EFS-Alsace


	(1) PC (100% plasma) 1/1/2003 – 1/2/2004 99.6 %	(2) PC (35% plasma+65% T-Sol) 1/9/2005 – 1/6/2006 95 %	(3) PC INTERCEPT (35% plasma+65% Intersol) 1/9/2006 – 1/8/2007 99 %
Patients (n)	59	33	36
Adverse reactions (n)	67 (11 RBC Imm)	41 (16 RBC Imm)	37* (19 RBC Imm)
Adverse reactions/1000 PC (n)	5.3	2.7	1.4
Patients with reactions	2.9 %	2 %	1.7 %

Period 3 : *Fever/chills : 8 ; allergy : 3 ; TRALI : 1 ; RBC immunisations : 19

Period 1 : 1 death volume overload (2 RBCC + 2 BCPC)

All 3 periods : No bacterial sepsis

All 3 periods : 145 adverse reactions : SEVERITY grade 1 = 61 %; grade 2 = 33 % (46 RBC immunisations)

BioOne Corporation
IMPUTABILITY grade 2, 3, 4 = 87 %; grade 3 and 4 = 70 % 

La Reunion (フランス)

EFS-La Réunion : Adverse reactions with INTERCEPT treated Apheresis PC -1

(M F Angelini-Tibert and P. Rasongles)

year	2005	2006/3 - 2007/3
nb of patients	NA	427
nb of apheresis PC	1194	1948
nb of adverse reactions	109	19
Adverse reactions / 1000 APC	91,3	9,8

3. EU主要国における不活化技術導入後不要になった技術(白除、NAT等)

国	不要になった技術
ベルギー	細菌検査、 γ 線照射、CMV検査、導入予定の個別NATの導入中止
フランス	γ 線照射、導入予定の個別NATの導入中止
ドイツ	γ 線照射
イタリア	
スペイン	
スウェーデン	
ノルウェイ	
ロシア	

東欧のIBS導入センターでは導入予定の白除の導入中止を検討しているが、まだ決定していない。

4. EU主要国における不活化技術を適用している製剤の市販後調査の実態(調査数)

1) 血小板

ヘモビジランス① (2003年10月～2005年12月)

ベルギー、イタリア、スペイン、ノルウェイ

輸血数 5106PC、患者数 651人、1人当たりの輸血数:1～156回 平均 7.8±16.2回

97.3%の血小板製剤はγ線照射なし

輸血関連の有害事象(可能性がある、おそらく関連、関連ある)

グレード1:41輸血、グレード2以上:1輸血 (全有害事象 42例、0.8%)

感染、TRALI及び死亡はない。

ヘモビジランス② (2005年5月～2007年1月)

ベルギー、フランス、スペイン

輸血数 7437PC、患者数 1400人、1人当たりの輸血数:1～129回 平均 5.3±10.8回

98.9%の血小板製剤はγ線照射なし

輸血関連の有害事象(可能性がある、おそらく関連、関連ある)

グレード1:55輸血、グレード2以上:0 (全有害事象 55例、0.7%)

感染、TRALI及び死亡はない。

ヘモビジランス③ フランス(Ile de La Reunion)

Ile de La Reunion では2005年から2006年にかけてチキングニャが大流行し島内採血を禁止した。急遽、本国で導入されているIBS不活化技術を導入後、島内採血を再開し血小板を確保した。

輸血数 1948PC、有害事象 0.98%

チキングニャ感染例はない

ヘモビジランス④ フランス(Alsace) (2006年9月～2007年8月)

輸血数 13241PC、患者数 2069人、1人当たりの輸血数:1～289回 平均 6.4回

有害事象 37輸血(0.14%)

TRALI 1例、感染、死亡は報告されていない。TRALI は出産経験が3回ある女性の成分献血による。

ヘモビジランス⑤

輸血数 4000PC 2008年6月 International society of blood transfusion で発表予定

ヘモビジランス⑥ ドイツ

輸血数 5000PC 2008年より開始

2) 血漿

ヘモビジランス①

輸血数 3000PC 2008年6月 International society of blood transfusion で発表予定

なおバイオワンとシーラスでは Hemovigilance に当たる市販後調査を継続して実施する予定です。アジアにおいても欧州と同じ基準により実施しますが日本は日本赤十字社のご判断によると思います。