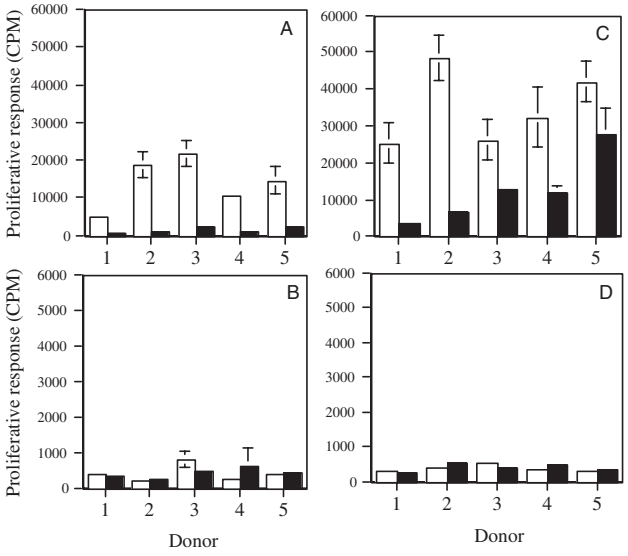

Fig. 1. Proliferative responses of Mirasol PRT–treated and untreated donor cells to allogeneic or xenogeneic stimulator cells. Five different paired sets of MNCs that had received Mirasol treatment (B and D) or no treatment (A and C) as responders were incubated with mitomycin C–treated allogeneic PBMNCs (A and B, □) or xenogeneic C57BL/6 spleen cells (C and D, □) or medium alone (■) for 5 days, and [^{3}H]thymidine incorporation was measured on Day 5.

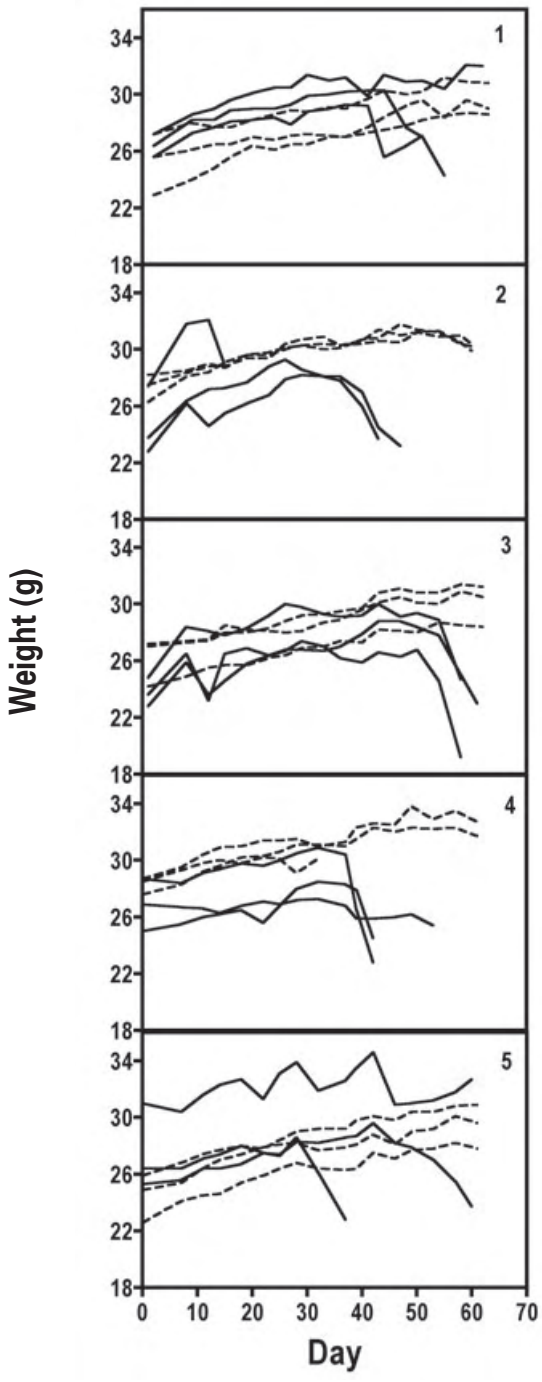

Fig. 2. The weight of recipient mice injected with untreated or Mirasol-treated donor cells. The weights (g) of recipient mice (3 mice per group) injected on Day 0 with Mirasol-treated (- - -) and untreated (—) cells from each of five donors (Panels 1-5).

of human cell chimerism (Table 1). In addition to exhibiting human cell chimerism, the recipients injected with untreated donor cells also exhibited increased spleen weight (splenomegaly) and significantly decreased Hct levels (Table 1).

In the cell populations from the recipient mice that contained sufficient numbers of human CD45+ cells to analyze, samples of cells were stained with a panel of antibodies to define the distribution of various WBC subpopulations in the lymphoid compartments. The results (Table 2) demonstrated that human T cells were the cells that were primarily present in the spleen, blood, and marrow. B cells were primarily found in the liver and intestinal lymphoid tissue. With the exception of one recipient mouse in which CD56+ cells were observed, no reconstitution with CD14+ cells (macrophages) or CD56+ cells (NK cells) was observed in any lymphoid compartment.[4] The cells were also stained with antibodies to CD4 and CD8 and the ratio of the percentage of T cells that were CD4+ to the percentage of T cells that were CD8+ T cells was determined. The CD4:CD8 ratios in the recipients of untreated cells from the different donors varied from 0.38 ± 0.08 to 1.82 ± 0.8 in a donor-dependent fashion.

Acute GVHD and especially TAGVHD are characterized by cytolytic responses. To determine whether the human T cells found in these mice exhibited properties of cytolytic cells, the T-cell subsets from a subset of recipients were dual-stained with anti-CD107a, a marker of cells that have previously mediated exocytosis of cytotoxic granules.[14,15] This staining procedure identified 41.1 ± 16.7 percent of human CD4+ cells (n = 8) and 45.5 ± 20.2 percent of human CD8+ cells (n = 8) as expressing CD107a. This would suggest that both CD4+ and CD8+

TABLE 1. Characterization of immune responses and reconstitution in mice injected with untreated or Mirasol PRT–treated MNCs

Parameter	Untreated donor cells	Treated donor cells
Number of mice with GVHD symptoms	12/14*	0/14*
Day euthanized	51.8 ± 8.2 days	Not applicable†
Number of mice with human cells	12/13‡	0/14
Spleen weight (gram)	0.27 ± 0.27	0.07 ± 0.07
Hct (%)	27.9 ± 16.9	53.9 ± 2.9
Percentage of CD45+ cells in		
Spleen	26.8 ± 19.8	0.0
Blood	4.6 ± 4.7	0.0
Marrow	4.9 ± 5.6	0.0
Intestinal lymphoid tissue	58.4 ± 28.4	0.0
Liver	20.3 ± 20.7	0.0

* One mouse was lost from a total of 15 recipient mice for unrelated reasons as detailed under Materials and Methods.
† None of the recipient mice in this group exhibited GVHD symptoms so mice were euthanized at the end of the experiment.
‡ One mouse that exhibited GVHD symptoms died before analysis could be conducted.

TABLE 2. The human lymphocyte subpopulations present in recipient lymphoid compartments

		Total number of human CD3+/CD19+ cells ($\times 10^6$)*				
Donor	Mouse	Spleen	Blood	Marrow	Liver	Intestinal
1	4	65.0/0.0	0.3/0.0	5.4/0.0	3.2/8.8	0.0/0.0
1	5	0.0/0.0	0.0/0.0	0.0/0.0	0.0/0.0	0.0/0.0
1	6	0.4/0.2	0.01/0.06	0.03/0.4	1.5/6.1	0.7/0.2
2	10	2.8/0.02	0.04/0.1	0.1/0.2	0.2/1.1	0.1/0.5
2	12	1.5/0.4	0.2/0.6	0.7/0.7	0.3/1.2	0.7/5.3
3	16	0.08/0.4	0.06/0.07	0.1/0.2	0.2/2.1	0.0/0.0
3	17	0.09/0.4	0.04/0.06	0.1/0.1	0.5/2.7	0.04/0.4
3	18	0.04/0.01	0.02/0.1	0.08/0.1	0.1/0.5	0.0/0.0
4	22	1.3/0.4	0.1/0.02	0.2/0.1	2.0/6.6	0.4/0.01
4	23	4.2/0.3	0.2/0.01	0.2/0.01	0.0/0.0	0.3/0.01
5	28	5.7/0.7	0.5/0.5	0.9/0.09	5.5/5.3	0.5/0.6
5	29	3.5/0.2	0.2/0.05	1.8/1.0	0.0/0.0	0.1/1.0
5	30	4.3/0.09	0.3/0.0	0.4/0.0	0.4/1.1	0.0/0.0

* Each number is the product of the cell recovery in millions for each lymphoid compartment and the percentage of human CD3+ or CD19+ detected by flow cytometric analysis in each population.

cells have mediated cytolytic activity via granule exocytosis. CD4+CD25+ have been recently shown to contain granzymes and mediate cytolytic activity against autologous cells.[16,17] Staining of a limited number of samples with anti-CD25 found that less than 5 percent of the CD4+ cells expressed CD25, suggesting that the human CD4+ cells present in the spleen were not T regulatory cells.

Measurement of the levels of human cytokines in the plasma of the recipient mice was used as another approach to assess the possibility that human cells were present but not located in the lymphoid compartments. The plasma samples from recipient mice that had been injected with Mirasol PRT–treated cells did not contain any human cytokines. In contrast, the plasma samples of mice injected with untreated donor cells (n = 13) contained very high levels of interferon-γ (IFN-γ); high levels of interleukin (IL)-10; low levels of IL-5, IL-1β, tumor necrosis factor-α (TNF-α), IL-8, and IL-12p70; and no IL-2 or IL-4 (Fig. 3).

The measurement of human immunoglobulin levels in the plasma was a third approach to measure the presence of human lymphoid cell chimerism. An ELISA to measure the levels of human IgG and IgM in the plasma of the recipient found that human immunoglobulins were only detected in the plasma of recipients injected with untreated cells (Table 3). To test for the specificities exhibited by the human immunoglobulin, C57BL/6 spleen cells were stained with a 1:10 dilution of the recipients' plasma followed by fluorescein isothiocyanate–labeled anti-human IgM or IgG. Flow cytometric analysis of WBCs, RBCs, and PLTs was conducted by gating on the different sized populations. The results of this experiment (Table 3) indicated that there were high levels of IgM antibodies and lower levels of IgG antibodies binding to murine RBCs and PLTs with very low levels of antibodies binding to WBC.

DISCUSSION

Mirasol PRT is a novel technology for pathogen reduction that has been shown to effectively preserve PLT viability in a PLT recovery and survival clinical trial.[18] The validation clinical trial for its efficacy and safety in thrombocytopenic patients has just been initiated in Europe. During Mirasol PRT treatment, blood products such as PLT concentrates are exposed to light in the presence of riboflavin resulting in inactivation of a wide range of pathogens.[19-23] Because this process introduces irreparable lesions on nucleic acids,[20] the treatment was also expected to affect the function of WBCs. In a previous study, it was shown that Mirasol PRT treatment of WBCs prevented their ability to be activated, to proliferate in response to various stimuli, and to induce proliferation of normal PBMNCs.[11] The conclusion of these studies was that Mirasol PRT treatment caused a complete functional inactivation of WBCs. Several studies had previously shown that inhibition of in vitro proliferative responses correlated with an inability to generate GVHD.[13,24] To confirm the inactivation of WBC by Mirasol PRT treatment observed with in vitro assays also prevented in vivo GVHD responses, the ability of Mirasol PRT–treated cells to induce xenogeneic GVHD responses when injected into Rag2$^{-/-}\gamma c^{-/-}$ double-knockout recipient

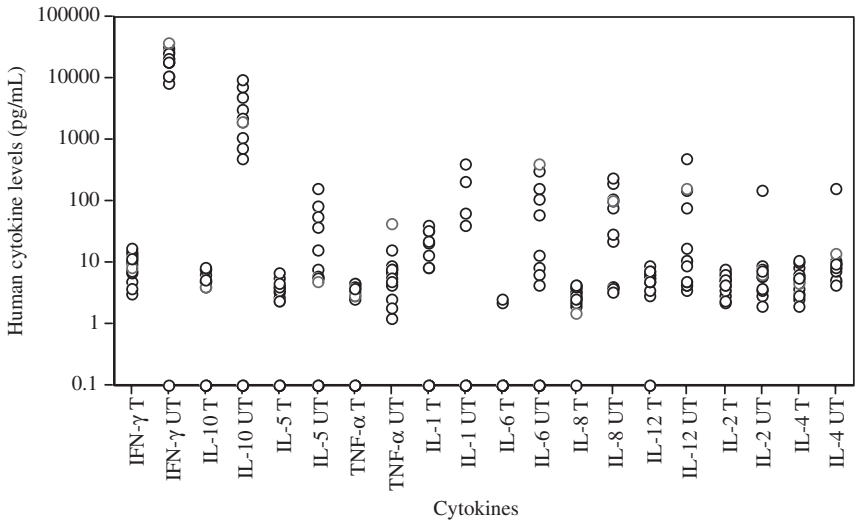

Fig. 3. Human cytokine levels in recipient mice plasma. Plasma samples obtained from recipient mice injected with Mirasol-treated cells (T) or untreated cells (UT) were tested for the concentrations of the indicated cytokines. The data show the results when undiluted plasma was tested except for the 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 ± 2781*	0.1 ± 0.2*
IgM	Not tested	1390 ± 845*	0.3 ± 0.8*
IgG	Mouse RBCs	16.1 ± 17.9†	0.3 ± 0.4†
IgM	Mouse RBCs	30.8 ± 21.7†	0.2 ± 0.5†
IgG	Mouse WBCs	5.1 ± 6.1†	0.5 ± 0.3†
IgM	Mouse WBCs	8.9 ± 7.3†	0.2 ± 0.4†
IgG	Mouse PLTs	28.2 ± 17.8†	0.3 ± 0.6†
IgM	Mouse PLTs	25.6 ± 23.5†	0.0 ± 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 PBMNCs in an MLC assay.[11] This would suggest that Mirasol PRT treatment 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$^{-/-}\gamma c^{-/-}$ double-knockout recipient mice. A previous study had shown that injection of human PBMNCs into Rag2$^{-/-}\gamma c^{-/-}$ double-knockout recipient mice resulted in enhanced peripheral reconstitution with human T cells.[4] Separation of donor PBMNCs 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$^{-/-}\gamma c^{-/-}$ double-knockout recipients found high levels of IL-1β, 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-1β, 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 $Rag2^{-/-}\gamma c^{-/-}$ 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 $Rag2^{-/-}\gamma c^{-/-}$ 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 $Rag2^{-/-}\gamma c^{-/-}$ 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.

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TRANSFUSION MEDICINE REVIEWS

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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.

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

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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 rates[1] 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 Prevention[11] 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 psoralens[12] and riboflavin[13] 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	*Treponema pallidum*, *B burgdorferi*	>5.9 to >10.6
Protozoa	*Plasmodium falciparum*, *T cruzi*, *B microti*	>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.

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