The Rationale and Use of Platelet Concentrates Irradiated With Ultraviolet-B Light

Derwood H. Pamphilon

ULTRAVIOLET-B (UVB) irradiation has profound immunomodulatory effects and modifies a number of interactions within the immune system. These include impaired cutaneous immune responses such as contact hypersensitivity (CHS) and cellular antigen presentation by specialized cells such as Langerhans cells and dendritic cells. In clinical practice, the immunization to human leukocyte antigens (HLA) that may follow transfusion can be manifest as refractoriness to randomly selected transfusions of platelets. Data show that transfusion of UVB-irradiated platelet concentrates (PCs) reduces HLA immunization and platelet refractoriness when compared with transfusion of unmodified PCs. UV-B irradiation prevents the interaction of dendritic cells contained in transfusates with recipient T lymphocytes, which may be central to prevention of HLA alloimmunization. In addition, there is evidence that failure of cooperation between donor and recipient immune cells may induce a state of tolerance after transfusion. In support of this, data from solid organ transplantation studies in animal models indicate that either UVB-irradiation of donor-type transfusions given pretransplantation or in the case of certain tissues such as pancreatic islets direct illumination of the graft may induce tolerance and promote graft acceptance.

Transfusion of cellular blood products is associated with a number of adverse effects, including alloimmunization, febrile nonhemolytic transfusion reactions (FNHTR), viral and bacterial transmission, and transfusion-associated graft-versus-host disease (TA-GVHD). Longer-wavelength UVA light causes cell damage in a number of ways and in conjunction with photosensitizing agents modifies DNA and RNA via the formation of photoadducts with DNA and RNA crosslinking. UVA light and photoactivation have been used to inactivate viruses and bacteria by preventing their replication and to prevent both lymphocyte proliferation and gene transcription, leading to cytokine synthesis. Shorter-wavelength UVC and UVB also modify nucleic acid via the induction of pyrimidine dimers. The action spectrum for the latter is maximal at 254 nm (UVC/UVA). In animal models, T-lymphocyte inactivation by UVB has been shown to prevent GVHD. It is unclear whether UVC/B light could also prevent FNHTR and inactivate viruses and bacteria at doses that are not damaging to platelet function.

This review summarizes data on UVB irradiation and its utility in transfusion practice, addressing the following issues: how are alloreactive responses inhibited by UVB irradiation; is UVB irradiation feasible in the routine production of blood components; can UV irradiation confer clinical benefit by reducing or preventing alloimmunization, FNHTR, TA-GVHD, and transfusion-transmissible infections (TTI); finally, can adverse transfusion outcomes be prevented more effectively by other approaches such as leukodepletion and gamma irradiation.

PHYSICAL EFFECTS OF ULTRAVIOLET LIGHT

UV irradiation is divided by wavelength into UVC (200 to 280 nm), UVB (280 to 320 nm), and UVA (320 to 400 nm). UVC is approximately 10 times more effective than UVB and 10^5 times more effective than UVA when assessed by its effect on cell viability. UVC irradiation is absorbed in the atmosphere, and very little, if any, reaches the earth. It is germicidal and causes erythema. UVB and UVA irradiation are the major carcinogenic components of sunlight, and UV irradiation is responsible for melanin pigmentation. All UV irradiation causes cell death, UVA predominantly by exerting oxidative stresses, causing cell membrane damage and specific types of DNA damage, mainly single-strand breaks and protein-DNA crosslinks. When photosensitizing agents such as psoralens are used in conjunction with UVA, the psoralens bind irreversibly to nucleic acid and form

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monoadducts and interstrand crosslinks with DNA and RNA when illuminated. By contrast, UVB/C induce the formation of cyclobutane pyrimidine dimers and pyridimidine (6-4) pyrimidone dimers, which block elongation of nucleic acid transcripts. Cells attempt to repair such defects by excision of nucleic acid lesions—transcription coupled repair (TCR). However, after a lethal dose of UV-irradiation, cells undergo apoptosis. Such programmed cell death is regulated by a number of genes, including the tumor suppressor gene p53, which has 2 principal actions. First, it arrests cycling cells at the G1 to S-transition to facilitate repair, and second, it allows cells to undergo apoptosis by transcriptional activation or inhibition of genes, including bax and bcl-2. At lower dose levels, the effects of UVB light are more subtle. Such irradiated cells may continue to function (eg, platelets, pancreatic islets). Modification of lymphoid cells can induce tolerance, and the presence of UVB-irradiated leukocytes in blood used for transfusion may in some way deliver a tolerogenic signal.

The signaling pathways involved in T cell activation are shown schematically in Figure 1. Antigen presented in the groove formed by class II HLA interacts with the T cell receptor. Contact between the antigen-presenting cell (APC) and the T cell is stabilized by CD4 and by the interaction between LFA-1 and CD54 (ICAM-1). This first, or antigenic, signal induces the upregulation of CD40 ligand on T cells. The second or costimulatory signal is initiated by the binding of CD40 ligand to CD40 on dendritic cells, which upregulate their expression of CD80 and CD86. These molecules link with CD28, leading to T-cell activation. The production of interleukin (IL)-12 by dendritic cells during this sequence of events is crucial in driving T-cell proliferation and secretion of IL-2. It is known that if an antigenic signal is not followed by a second costimulatory signal, then anergy or tolerance may result. A leading hypothesis contends that after UV-B irradiation, limited signaling between MHC class II—bearing dendritic cells and T cells is still possible, whereas second signal interactions are not.

Cell Surface Ligands and Cytokines

It has been shown that HLA-A, -B, and -C antigens could still be defined serologically after shortwave UV irradiation (100 to 120 J/m²). Although HLA-DR could be defined serologically, more recent reports have shown that MHC class II antigens are reduced after UVB irradiation in a manner that is both time and dose dependent; HLA-DQ and -DP antigens are more sensitive than -DR, despite the fact that the former are expressed at a higher level on dendritic cell membranes. Data also show that T-cell activation is dependent on the
expression and function of a range of critical cell surface ligands (see above) and the production of specific cytokines. UV irradiation seems to affect cytokine production by a number of cell types, and the effects observed vary according to wavelength and dose. It is now known that there are 2 types of CD4 (T-helper) cells: Th-1 cells secrete IL-2 and gamma interferon, causing CD8-mediated cytotoxicity, and Th-2 cells that secrete IL-4 and IL-5, which induce B cells to proliferate. Experiments show that either IL-12 or IL-10 production may occur after UV irradiation and that this, in turn, determines whether Th-1 or Th-2 responses, respectively, are induced.

Costimulatory signals are blocked partially when activated accessory cells are incubated with an antibody to CD54. The expression of CD54 and CD14 on monocytes is reduced in a dose- and time-dependent manner after UVB-irradiation, although expression on B lymphocytes is largely unaffected at the same doses. LFA-1 expression on T lymphocytes is reduced after UVB irradiation. There is disagreement as to the effect of UV light on CD80 and CD86 molecules. In one study, immune suppression in mice, including generation of suppressor T cells and systemic impairment of APC function, was blocked by monoclonal anti-CD86 but not by anti-CD80, suggesting that UVB irradiation favored costimulation by CD86. Such costimulation leads to production of IL-10 and suppression of Th-1-type immune reactions. However, in another study, it was shown that UVB irradiation of Langerhans cells decreased the expression of CD86 and CD54, but not CD80. Moreover, UVB irradiation inhibited MLC responses, and the effect was partially reversed by addition of exogenous anti-CD28 monoclonal antibodies.

Cell surface antigens are in continuous flux. Shed antigens are replenished from intracellular stores. It is possible that the differential effects of UVB exposure on antigens results from differences in intracellular synthetic and recycling mechanisms.

Most studies describing the effect of UV irradiation on cytokine secretion have been performed in models of cutaneous UV irradiation, where a number of immune responses such as CHS are impaired. UV irradiation causes a reduction in mRNA for IL-1 receptor but increases mRNA for IL-1 and IL-6. The effect is maximal at 254 nm (UVC), but little activity is seen at >313 nm (UVB). This action spectrum is similar.

Fig 1. The role of CD40-CD40L in T-cell activation. The figure depicts a 2-step model of activation of T cells. (A) Step 1: Induction of CD40L on T cells. Antigens are taken up by unprimed naive dendritic cells (DC) (Langerhans cells in tissues). These DC migrate to the LN, where they present processed antigen to naive T cells in the form of MHC-peptide complexes and deliver the antigenic signal (signal 1) via the TCR to naive T cells. As a result, T cells upregulate CD40L on their surface. (B) Step 2: Induction of costimulatory activity on DCs. CD40L on the surface of T cells induces costimulatory activity on DCs via the CD40-CD40L interaction. The primed APC-expressing costimulatory molecules sends a second costimulatory signal to T cells along with signal 1 for full activation of T cells to produce cytokines and to perform immune effector functions. Abbreviations: APC, antigen-presenting cell; DC, dendritic cell; IL-12, interleukin-12; LN, lymph node; MHC, major histocompatibility complex; TCR, T-cell receptor. Taken from Grewal and Flavell, Immunology Today 17:410-414, 1996, with permission.
to the effect of UV irradiation on the induction of cyclopyrimidine dimers in nucleic acid. Studies employing UVB irradiation of transformed human dermal microvascular endothelial cells (HMEC) show an increase in mRNA and release of IL-1β, IL-6, and IL-8. HMEC are an important target for study because they recruit inflammatory cells to the skin after UV irradiation. It appears that UVB irradiation increases translation and release of IL-6 and IL-8 via the action of IL-10. It has been shown in models of CHS that UV-treated murine cells can transfer suppression to secondary animals. This effect is abolished if the primary UV-irradiated mice are injected with IL-12. Such disruption of UV-induced tolerance probably occurs through inactivation or inhibition of CD8+ suppressor T cells. Paradoxically, when keratinocytes are irradiated with UVA, levels of IL-12 are increased with subsequent activation of Th-1 cells and prevention of Th-2 cell generation. By contrast, UVB irradiation increases IL-10 secretion and is therefore more likely to inhibit normal cutaneous immune responses by inhibiting Th-1 cells and facilitating the proliferation of Th-2 cells. Additional evidence from IL-4 knockout mice suggests that this cytokine also has an important role in suppression of immune responses. It can therefore be postulated that cutaneous UV irradiation causes the release from keratinocytes of cytokines such as IL-10, which facilitate the proliferation of Th-2 cells, leading to the suppression of cytotoxic immune responses. However, the effects of UV irradiation are not restricted to areas of cutaneous erythema. For example, splenic APCs are known to have impaired function in mice, where limited areas of local erythema are induced. In addition, it has been shown that the supernatant from irradiated keratinocytes injected intravenously into naive mice inhibits splenic APC function. Restoration of normal cellular function has been observed after treatment with anti-IL-10. This finding suggests that UVB irradiation facilitates the generation of IL-10 and reduces production of IL-12, the latter critical to T cell activation after interaction with dendritic cells, whereas the former impairs APC function. UVB irradiation also induces the secretion of other cytokines such as IL-1β, IL-6, and IL-8, which are pro-inflammatory.

Altered APC function in Langherhan and dendritic cells after higher doses of UVB irradiation may be due, in part, to DNA damage with the formation of cyclopyrimidine dimers. This is suggested by experiments in mice given limited cutaneous UVB exposure where APC from draining lymph nodes were mixed with a liposome-containing photolyase and then exposed to a further photoactive stimulus. The treatment allows for splitting of cyclopyrimidine dimers, after which restoration of APC function was observed. The findings were not observed if photolyase was used alone or if the photoactive stimulus was applied after the initial cutaneous UV stimulus.

**IMMUNOLOGIC EFFECTS OF UVB IRRADIATION IN VIVO**

It is generally accepted that after transfusion there are two mechanisms of alloantigen presentation that may lead to recipient alloimmunization to HLA. The direct route requires donor (transfused) dendritic cells to present their own HLA class I by direct contact with recipient lymphocytes via the pathways described. Alternatively donor leukocytes or platelets may be processed by recipient dendritic cells (indirect route), leading to presentation of donor HLA to recipient T cells. There is substantial in vivo evidence that the direct pathway is of primary importance in recipient alloimmunization to foreign HLA. Leukoreduced PCs were not able to induce an immune response to histocompatibility antigens in a rodent transfusion model, whereas contamination with a small number of donor strain leukocytes restored the response. When dogs receive marrow grafts from dog leukocyte antigen-identical littermates 98% are accepted, although there is uniform graft rejection if pretransplantation transfusions from the donors are given. When the transfusions are first irradiated with UV-C, graft failure does not occur. However, if fresh nonirradiated dendritic cells are also transfused, there is once again uniform graft rejection. Dendritic cells are extremely sensitive to the effects of both UV irradiation and temperature compared with other accessory cell populations. There is permanent graft acceptance when ACI cardiac allografts are transplanted into histo-incompatible Lewis recipients if UV-irradiated donor-strain transfusions are given pretransplantation. After transplantation, lymphocytes from the recipients show reduced responses in MLC to donor strain splenic lymphocytes, and the infusion of 10^4 donor-type
dendritic cells at 40 or 60 days after transplantation leads to acute allograft rejection. Together, these data suggest that UV-induced inhibition of donor dendritic cells prevents a stimulatory interaction with recipient allogeneic lymphocytes that may be of central importance in the induction of alloimmunization. Conventionally, most foreign antigens are processed and presented by dendritic cells. Given that platelets express abundant class I HLA and that pretransfusion removal of donor leukocytes to less than $5 \times 10^6$ per transfusion of PCs prevents HLA sensitization in more than 97% of previously nontransfused or pregnant patients, the indirect pathway must be of secondary importance.

### PRACTICAL CONSIDERATIONS IN UVB IRRADIATION

UVB dose is the product of the intensity of the source (watts/m$^2$) and the time taken for irradiation (secs). The product is usually expressed as Joules per square meter. Many reports seem to have shown similar biological effects after exposure to a wide range of UVB doses. This is almost certainly the result of using different methodologies, which measure irradiance either at the peak wavelength or throughout the spectrum of activity (integrated dose). In our studies, we used UVB measured at a dose of 3,000 J/m$^2$ at the peak wavelength of 310 nm. When expressed as the integrated dose, it was calculated to be 22,500 J/m$^2$. Permeability to UVB irradiation varies considerably from plastic to plastic. Mostly the polyolefin and polyvinyl chloride plastics commonly used in the manufacture of platelet packs are poorly UVB-permeable. Available reports show that certain polyolefin plastics, such as the Stericell pack (DuPont, Stevenage, UK), PL 269 (Baxter, Round Lake, IL), ethylene vinyl acetate (EVA) packs such as the EVA-Macopharma (Macopharma, Tourcoing, France), and Teflon (American Fluoroseal Corp., Silver Spring, MD), have reasonable transmittance (Table 1). To ensure consistency when irradiating PCs with UVB, it is important to standardize the cross-sectional depth of the pack. A cross-sectional depth of 5 mm or less can be achieved by regulating the volume of the PC. Cooling devices are also required in some UVB irradiation cabinets because most UV sources also produce infrared light, which can result in considerable heat gain.

### Table 1. Transmission (%) of UVB Irradiation Through Various Plastics

<table>
<thead>
<tr>
<th>Plastic</th>
<th>310 nm</th>
<th>302 nm</th>
</tr>
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<tbody>
<tr>
<td>Cutter CLX</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>Biotest 5 day</td>
<td>29</td>
<td>—</td>
</tr>
<tr>
<td>Terumo 5 day</td>
<td>17</td>
<td>—</td>
</tr>
<tr>
<td>Fenwal PL 146</td>
<td>17</td>
<td>20</td>
</tr>
<tr>
<td>Fenwal PL 732</td>
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<td>34</td>
</tr>
<tr>
<td>Fenwal PL 269</td>
<td>—</td>
<td>62</td>
</tr>
<tr>
<td>DuPont Stericell</td>
<td>75</td>
<td>—</td>
</tr>
<tr>
<td>Fluoroseal Teflon</td>
<td>—</td>
<td>86</td>
</tr>
</tbody>
</table>

**NOTE.** All measurements made through a single thickness of plastic. Modified from Pamphilon DH, Blundell EL: Semin Hematol 28:113-121, 1992, with permission.

### IN VITRO EFFECTS OF ULTRAVIOLET LIGHT ON PLATELETS

Platelet ultrastructure is affected by storage under standard conditions. The tubulocannalicular system shows dilation, and there is a loss of dense bodies, giving rise to a vacuolated appearance. Disc-sphere transformation is also apparent. We did not find that these changes were accentuated after irradiation with UVB (3,000 J/m$^2$). By contrast, shortwave (254 nm) UV irradiation was noted to cause pseudopod formation at the platelet surface. It is probable that platelet ultrastructure is adversely affected by escalating doses of UV irradiation, but data from systematic studies at different wavelengths are not available. At a higher dose of UVB (100,000 J/m$^2$), the morphology score (Kucicki) of treated and control PCs was comparable 1 day after irradiation but was significantly worse among treated cells 4 days after irradiation. It was also observed that escalating doses of UVB irradiation cause small platelet aggregates that may be visible macroscopically. When platelet samples are collected into citrate anticoagulant or into ethylenediaminetetra-acetic acid (EDTA) with or without formalin, the presence of small aggregates was noted by a fall in the platelet count in the presence of formalin. In one report, stirring PCs resulted in a larger number of platelet aggregates. The effect was most marked at 60,000 J/m$^2$, using Westinghouse FS-20 lamps (range, 280 to 320 nm) and at 120,000 J/m$^2$, using Phillips TL01 lamps (312 to 313 nm). In addition, twice the UV dose from the TL01 sources was required to reduce responses in MLC to less than 10%. Thus, there appears to be no particular benefit in using the narrow waveband source, which eliminates the small amount of
coincident UVC irradiation found in broad-spectrum UVB lamps such as the FS-20 and the TL12 lamps. Platelet aggregation is known to be fibrinogen-dependent and to require calcium. Fibrinogen binds to the platelet glycoprotein (GP) IIb/IIIa, which may undergo a conformational change during activation. After irradiation with 100,000 J/m², the dose also shown to impair platelet morphology scores, the expression of immunoreactive GP Ib was shown to decline to 60% of its original value by 4 days. We have shown that the release of glycocalcin (GC—a major segment of GP Ib essential for platelet adhesion) increased in the supernatants of UVB-irradiated PCs. We evaluated 2 different UVB sources and looked at the effect of dose-escalation and the length of storage after irradiation. GC release was significantly higher with increasing UVB doses and at day 5 when compared with day 1. Higher differential GC release was also shown to correlate with a decrease in hypotonic shock response (HSR) and an increase in lactate and β-thromboglobulin (TG) levels, suggesting that elevated levels were indicative of a greater platelet storage lesion after UVB irradiation.

We have performed a variety of additional studies of platelet function during storage after UVB irradiation with 3,000 J/m² in Stericell containers and compared our results with those from control, nonirradiated PCs. The dose was selected because it had previously been shown to inactivate lymphocyte reactivity in MLC. Our principal findings were that there were no differences in pH, HSR, or aggregation responses to adenosine diphosphate (ADP), collagen, epinephrine, or ristocetin. However, a significant increase in lactate concentration occurred through 5 days of storage. Other investigators have shown variable effects on platelet aggregation after irradiation with 30,000 to 60,000 J/m² at a mean wavelength of 310 nm (Table 2). When PCs were irradiated with 100,000 J/m², there was a drop in pH and morphology score at 4 days. Although platelet aggregation responses were not reported, platelet metabolism was studied by measurement of plasma concentrations of ATP, ADP, lactate dehydrogenase, ammonia, glucose, glutamine, glutamate, hypoxanthine, pO₂, and pCO₂. None of these measurements showed a significant change among treated PCs compared with control PC maintained at room temperature or 37°C for 4 days.

Platelet activation has been studied by measuring the release of β-TG and platelet factor-4 (PF4). We found that PF4 levels were higher in UVB-irradiated PCs (TL12; 3,000 J/m²). Others found that the platelet content of serotonin, PF4, and thromboxane-B₂ was reduced after exposure to UVB doses of 80,000 J/m² (FS-20) or 160,000 J/m² (TL01). Taken together, these in vitro data indicate that PCs may be irradiated with UVB light at doses that inactivate residual leucocytes, but that preserve acceptable platelet function through 5 days of storage in suitable containers. However, at higher doses, platelets aggregate in a fibrinogen-dependent manner via GP IIb/IIIa, show loss of GP Ib, evidence of activation, and some signs of metabolic deterioration.

### IN VIVO EFFECTS OF ULTRAVIOLET LIGHT ON PLATELETS

Having ascertained that UVB-irradiated PCs showed acceptable in vitro function, 3 groups have reported studies undertaken to investigate in vivo recovery and survival in either normal volunteers or thrombocytopenic recipients. We performed a paired study in 8 volunteers, of UVB-treated (3,000 J/m²) versus control PCs stored for 5 days and then infused. Median recovery (39.5% > 37.0%), half-life (4.25 d > 4.11 d), and survival (7.67 d > 7.43 d) did not differ significantly between UVB-treated and control PCs, respec-

### Table 2. Aggregatory Responses of Platelets Irradiated With UVB Light

<table>
<thead>
<tr>
<th>Reference</th>
<th>Source and Mean Wavelength (nm)</th>
<th>UV Dose (J/m²)</th>
<th>Agonists Tested</th>
<th>Impaired Responses</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>NS 310</td>
<td>15-30,000</td>
<td>ADP, arachidonic acid</td>
<td>No</td>
</tr>
<tr>
<td>9</td>
<td>Spectroline XX-15B 310</td>
<td>30-60,000</td>
<td>Collagen, ristocetin, A23187, adrenaline</td>
<td>Yes—ADP, Collagen</td>
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<tr>
<td>24</td>
<td>Westinghouse FS-20 310</td>
<td>17,820</td>
<td>ADP, collagen, ristocetin</td>
<td>No</td>
</tr>
<tr>
<td>37, 38</td>
<td>Phillips TL 12 310</td>
<td>3,100</td>
<td>ADP, collagen, adenalin, ristocetin</td>
<td>No</td>
</tr>
</tbody>
</table>

Abbreviations: NS, not stated.

Modified from Pamphilon DH, Blundell EL: Semin Hematol 29:113-121, 1992, with permission.
Patients undergoing cardiopulmonary bypass surgery received filtered red cells and a single transfusion of pooled platelets, either standard or UVB-irradiated (1.5 J/cm²) from 12 donors. HLA antibodies were detected among 6 of 62 recipients of test PCs versus 5 of 39 recipients of control PCs (P = .62). In this report, it was shown that a dose of 0.5 J/cm² markedly induced the formation of thymidine dimers and abolished ex vivo alloreactive responses, leading the authors to speculate that immunization among recipients of irradiated PCs may have resulted from indirect presentation of donor antigens via host APCs.

Andreu and colleagues enrolled 142 patients into a prospective 3-arm study that randomized them to receive either standard nonirradiated platelets, leukocyte-depleted platelets, or UVB-irradiated platelets. All red cell concentrates were leukodepleted by filtration. To be evaluable, patients were required to have received a minimum of 10 transfusions in a 6-month period. Seventy-two evaluable patients were reported, and 7 of 20 (35%) control patients were found to have made HLA antibodies, compared with 3 of 26 (11.5%; P = .059) recipients of leukodepleted PCs and 2 of 26 (7.7%; P = .027) recipients of UVB-irradiated PCs. In another multicenter study, 52 patients with newly diagnosed leukemia, lymphoma, or other cause of marrow failure requiring platelet transfusion support were studied. Patients received red cells leukodepleted by filtration and pooled nonirradiated platelets or platelets irradiated with 7.5 to 15.0 J/m² UVB. The requirements for red cell and platelet transfusions in the UVB and control arms were similar, and there was no difference between the groups in the frequency of reported adverse effects after transfusion. Twenty-five percent of recipients of control PCs and 13% of recipients of UVB-irradiated platelets became alloimmunized, although this difference did not reach statistical significance. In subgroup analysis, it was noted that a larger number of women with prior pregnancies were enrolled in the UVB arm compared with the control group (12 v 5). This may have affected the results, as 3 of 4 patients who became alloimmunized after transfusion with UVB-irradiated platelets had prior pregnancies compared with 2 of 5 recipients of control platelets. The number of platelet transfusions given before sensitization was reduced in the UV group, as was the time to alloimmunization. One may speculate that a
greater difference between the rates of alloimmunization might have been observed had each study group contained equal numbers of previously pregnant women. To analyze this further, the data were reevaluated, considering only patients who had received more than 10 platelet transfusions. This analysis demonstrated a protective effect of UVB treatment, albeit of marginal significance ($P = .067$). The occurrence of refractoriness in both groups of patients was similar.46

Recently, the results of a large multicentered US study (Trial to Reduce Alloimmunization to Platelets—TRAP) have been published. 4 All patients had acute myeloid leukemia, were older than 15 years of age, received intensive chemotherapy, and were on study for 8 weeks. Recipients of multiple transfusions given 2 months or longer prestudy were ineligible, as were those who had received transfusions from more than 10 donors in the period 2 weeks to 2 months prestudy. Prophylactic platelet transfusions were, as in the studies aforementioned, given if the platelet count was less than 20 $\times$ 10$^9$/L. All patients received red cells filtered using the RC100 or BPF-4 (Pall, Medsep, Portsmouth, UK) filters ($<$5 $\times$ 10$^6$ WBC/unit). Patients were assigned to receive either pooled random donor PCs (PC), filtered random platelet pools (F-PC), prepared using PL100 filters (Pall, Medsep, Portsmouth, UK), UVB-irradiated random platelet pools (UVB-PC) irradiated with 14,800 J/m$^2$ of UVB in Stericell containers, or filtered random apheresis platelets (F-AP) collected using the COBE Spectra cell separator.

The primary study endpoint was the occurrence of alloimmune platelet refractoriness; secondary endpoints were refractoriness and alloimmunization. A total of 530 patients were enrolled. The age of the patients, male/female ratio, and the history of exposure to blood products or pregnancies were similar between the groups. Surprisingly, 80% of patients had received prior transfusions, and 81% of 244 women entered into the study had had a prior pregnancy. Therefore, only 12% of patients did not have an immunizing stimulus before enrollment into the study. The mean platelet dose was slightly lower in recipients of filtered products, but otherwise the number of transfusions given and the median donor exposure were equivalent for pooled products (the median donor exposure was roughly one-sixth for recipients of F-AP). It is remarkable that in a study of this size there was greater than 98% product conformance. Two percent of platelet transfusions were reported as being associated with severe adverse reactions, but specific information on FNHTR was not reported. The overall incidence of HLA antibodies was 45% of the control group and 17% to 21% in the 3 other study groups ($P < .001$). There was no significant difference between any of the intervention groups.

The overall incidence of alloimmune platelet refractoriness was 13% in the control group; this figure is low considering that 88% of patients enrolled in the study had either a prior pregnancy or transfusion. The incidence in the intervention groups varied from 3% to 5% ($P = .03$); there were no significant differences among the intervention groups. Ten percent of all patients met the study criteria for refractoriness (CCI $<$ 5 $\times$ 10$^9$/L after 2 consecutive ABO-compatible transfusions). Refractoriness occurred in 16% of controls compared with 7% to 10% of the intervention groups. In multivariate analysis, assignment to a treatment group significantly reduced the incidence of refractoriness, alloimmunization, and alloimmune platelet refractoriness, with the exception of UVB-PC transfusions and refractoriness ($P = .19$). Absence of prior pregnancy was a significant factor in all 3 study endpoints ($P < .001$). The authors concluded in this study that UVB irradiation and filtration were both effective in reducing the development of HLA sensitization and alloimmune platelet refractoriness. No additional benefit was seen from the use of single-donor apheresis platelets. 3

**Likely Impact of Different Wavelengths of Ultraviolet Light on Alloimmunization, FNHTR, TA-GVHD, and TTI**

UVA irradiation by itself has little biological effect, but in conjunction with the psoralen S-59, has been shown to achieve inactivation of viruses, bacteria, and leukocytes with good preservation of platelet function. 5,6 It therefore has the potential to minimize or prevent TTI and TA-GVHD (Table 3). The latter would be a useful development because the need for expensive gamma-irradiators would be obviated. UVA-S-59–treated leukocytes do not synthesize IL-8 and so cytokine-mediated FNHTR might also be prevented. Studies of the impact of these photochemically treated blood products on clinical efficacy (hemostasis, count increments, etc.), percent alloimmunization, FNHTR, and TA-
UVB-IRRADIATED PLATELETS

Table 3. Effect of Differing UV Wavelengths on Clinical Outcome

<table>
<thead>
<tr>
<th>Wavelength</th>
<th>HLA Alloimmunization</th>
<th>FNHTR</th>
<th>TA-GVHD</th>
<th>TTI*</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>UVA1</td>
<td>Not studied</td>
<td>Not studied, but IL-8 synthesis inhibited</td>
<td>Prevented in vitro and murine studies</td>
<td>In vitro inactivation of viruses and bacteria</td>
<td>5, 6</td>
</tr>
<tr>
<td>UVB</td>
<td>Reduced</td>
<td>Not studied</td>
<td>Prevented in murine and canine models</td>
<td>Not studied</td>
<td>8, 47</td>
</tr>
<tr>
<td>UVC</td>
<td>Reduced†</td>
<td>Not studied</td>
<td>Prevented in murine and canine models</td>
<td>Known to be germicidal. Shown to kill certain model viruses</td>
<td>8</td>
</tr>
</tbody>
</table>

Abbreviations: FNHTR, febrile nonhemolytic transfusion reaction; TA-GVHD, transfusion-associated graft-versus-host disease; TTI, transfusion-transmitted infection.

*In vitro studies only. Because the frequency of transmissible agents such as hepatitis C and HIV-1 is very low in screened blood products, comparative studies to show a significant reduction in TTI would require very large patient numbers.

†In conjunction with a photosensitizing agent, such as psoralen.

GVHD have not yet been reported. UVB irradiation can be delivered at doses that inactivate leukocytes with preserved platelet function through 5 days of storage. UVB-irradiated PCs reduce alloimmunization and platelet refractoriness, and animal studies show prevention of TA-GVHD. No information is available on FNHTR or TTI. UVC has not been the subject of clinical studies in humans (Table 3), and although it could inhibit all the adverse effects described, it may be excessively damaging to platelet viability and function. No further clinical studies of UVB or UVC have been reported or are known to be in progress.

RELATIVE MERITS OF ULTRAVIOLET LIGHT, LEUKODEPLETION, AND GAMMA-IRRADIATION

Gamma irradiation is used to prevent posttransfusion proliferation of the residual T lymphocytes contained in red cells and platelets. A dose of 2,500 cGy is used to inactivate T lymphocytes, and isolated reports suggest that TA-GVHD may occur after 1,500 to 2,000 cGy irradiation. Thus, the safety margin may not be wide. UVB/C irradiation inactivates T cells, and in vivo animal studies confirm that it can prevent not only GVHD after allogeneic bone marrow transplant (BMT) in histoincompatible donor-recipient pairs but also TA-GVHD after deliberate transfusion of a large inoculum of incompatible T cells. Similar observations have been reported after photochemical (UVA plus S-59) treatment of PCs. In addition, similar inactivation is seen with a 1,000× lower combined dose of UVA and psoralen, indicating a potentially much wider margin of therapeutic efficacy than with gamma irradiation.

Studies show that both leukodepletion and UVB irradiation are effective and equivalent in reducing recipient alloimmunization after platelet transfusion. Experimental data suggest that appropriate doses of UVA plus psoralen or UVB alone would also prevent or minimize FNHTR and TA-GVHD. TTI would be minimized after photochemical treatment, but viral and bacterial inactivation after UVB treatment has not been systematically studied. Furthermore, virucidal and bactericidal doses of UVB irradiation might cause an unacceptable acceleration in the development of the platelet storage lesion. There are data to show that leukocyte inactivation by UVB is less effective at higher leukocyte levels in a murine model of alloimmunization. One approach would be to photoinactivate leukodepleted PCs, although such a combined strategy has not yet been evaluated clinically. Finally, any new modification to blood components is of optimum value when applicable to all cellular components. Gamma irradiation inactivates leukocytes in both red cells and PCs, whereas there is no evidence that UVB or UVA plus psoralen are effective in red cell concentrates because hemoglobin exerts a quenching effect on the UV irradiation. Other strategies for red cell phototherapy, such as phthalocyanines plus red light, have compromised red cell integrity. Currently the most promising approach to inactivation of microbial agents and leukocytes in red cells appears to be the novel psoralen S-303, which functions in a light-independent manner. In the United Kingdom and other European countries, universal leukodepletion of the blood supply is being implemented. In the United Kingdom, universal leukodepletion was promoted because of theo-
retical concerns that the causative agent of new variant Creutzfeld-Jakob disease (nvCJD) might be transmitted by blood and its derivatives. Perhaps the optimum approach in future will be an initial leukodepletion step at the time of blood component preparation followed by phototherapy with UVB or UVA plus psoralen.

CONCLUSIONS

UVB irradiation damages nucleic acid, prevents DNA transcription, and ultimately compromises the integrity of the leukocytes contained within PCs. Experimental evidence indicates that UVB abolishes alloreactive responses and TA-GVHD. Clinical studies have shown a reduction in the rate of patient alloimmunization and refractoriness equivalent to that achieved by leukodepletion of platelets. UVB-irradiated platelets show in vitro, in vivo, and clinical evidence of functional equivalence to standard, nonirradiated PCs. The impact of UVB therapy on FNHTR and microbial transmission has not been evaluated. UVB and other photodynamic therapies appear promising in reducing adverse events associated with platelet transfusion.

ACKNOWLEDGMENT

The author thanks Bridget Hunt for her invaluable assistance in the preparation of this manuscript.

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